Cover Letter

SRA International, Inc.

December 30, 2009

Document Receiving Food and Drug Administration
Office of Food Additive Safety (HFS-255)
Center for Food Safety and Applied Nutrition, Food and Drug Administration,
5100 Paint Branch Parkway, College Park, MD 20740

Re: Dihydrocapsiate
GRAS Notification

Dear Dr. Robert Martin:

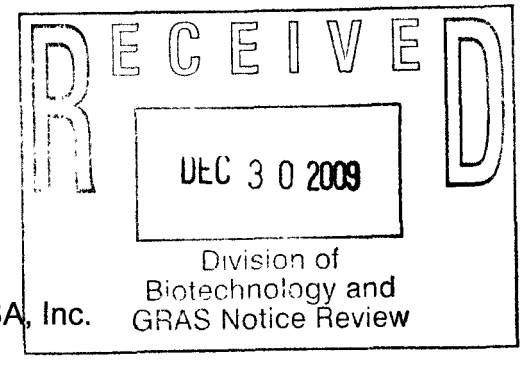
This document serves as the covering letter for a submission to the U.S. Food and Drug Administration (USFDA) Generally Recognized as Safe (GRAS) Notification process.

Attached please find three (3) copies of the submission for DIHYDRO CAPSIATE which has been declared GRAS by scientific procedure.

If you have any questions, please do not hesitate to contact me at the SRA offices.

Most Sincerely,

(b) (6)



Bruce K. Bernard, Ph.D.
President SRA International Inc.
Authorized Representative for Ajinomoto USA, Inc.

BKB/km Attachments 3

5235 Ragged Point Road, Cambridge, MD 21613-3518
Phone: 410.228.1400 Fax: 410.228.1450
Email: Bernard@sra-intl.com

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Notification

1 GRAS Exemption Claim

Ajinomoto USA, Inc., through its agent, SRA International, Inc., hereby notifies the Food and Drug Administration that the use of dihydrocapsiate described below is exempt from premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because Ajinomoto USA, Inc. has determined that such use is generally recognized as safe (GRAS) through scientific procedures.

(b) (6)

30 December 2009

Bruce K. Bernard, Ph.D.
President, SRA International, Inc.

Date

A. Name and Address of Notifier:

Ajinomoto USA, Inc.[1]
1120 Connecticut Avenue, Suite 1010
Washington, DC 20036, USA
202.457.0284 (Ext 301) (phone)
202.457.0107 (facsimile)
burseyb@ajiusa.com

Name and Address of Agent:

Dr. Bruce K. Bernard, President
SRA International, Inc.
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Cambridge, MD 21613
410.228.1400
bernard@sra-intl.com

[1] Ajinomoto U.S.A., Inc. is a subsidiary of Ajinomoto Company, Inc., Japan.

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B. Description of GRAS Approved Substance

The common name of the substance, the subject of this GRAS Notice, is dihydrocapsiate. The product, prepared by AJINOMOTO (AJ), is greater than 94% pure. Dihydrocapsiate is naturally occurring in a wide variety of edible, non-pungent, as well as pungent, chili peppers.

C. Intended Use and Consumer Exposure

Dihydrocapsiate is intended to be added to foods in a variety of food categories to provide 3 mg per standard serving, with the exception of chewing gum which would be enriched at a level of 10 mg/serving. The food categories in which the use of dihydrocapsiate is intended are presented in Table 1 below.

Table 1. Intended Use

Food Group	mg Dihydrocapsiate per Serving Size	FDA 21 CFR 101.12 Serving Size (g)[1]	Use Level (ppm)
Bars - Breakfast and meal replacement bars	3.0	40	75
Beverage concentrates[2] – low-calorie	3.0	21.6	138.9
Beverages soft drinks – sugar free	3.0	240	12.5
Chewing gun – sugarless	10.0	3	3333.3
Cookies – low fat and non-fat	3.0	30	100
Creamer	3.0	15 - 32	93.8 – 200
Energy drinks	3.0	240	12.5
Frozen Desserts – light	3.0		
Frozen desserts - ice pops and fruit bars	3.0	85	35.3
Frozen desserts – dairy	3.0	120 - 240	12.5 – 33.3
Fruit-aides, drinks, and powders – low calorie	3.0	240	12.5
Fruit juice – fresh orange juice	3.0	240	12.5
Gelatin/Puddings – low-calorie	3.0	120 - 147	20.4 – 25
Hard candy, dietetic (previously limited to mints)	3.0	2 - 40	75 -1500
Liquid coffee	3.0	240	12.5
Low-fat and non-fat crackers	3.0	30	100
Meal replacement beverages	3.0	30[3] - 240	12.5 - 100
Non-carbonated water – low calorie	3.0	240	12.5
Nutritional meal - Ensure	3.0	240	12.5
Oatmeal – instant, low sugar	3.0	240	12.5
Protein based meat alternative	3.0	7 - 240[3]	12.5 - 428.6
Ready to eat meals	3.0		
Ready to eat meals – frozen diet (non meat, non-chicken, non fish/seafood)	3.0	240	12.5
Ready to eat meals – soup	3.0	245	12.2
Ready to eat cereals, cold	3.0	15 - 55	54.5 - 200
Salad dressings – low-calorie	3.0	15 - 30	100 - 200
Snack foods – popped-low-calorie – low fat and rice cakes	3.0	30	100
Sweeteners – low-calorie	3.0	4	750
Tea, liquid low sugar or sugar fee	3.0	240	12.5
Vegetable juice	3.0	240	12.5
Yogurt – Fruit – Non and low Fat	3.0	225	13.3
Yogurt – Chocolate, Vanilla, Plain – Non and low fat	3.0	225	13.3

1 Title 21--Food and Drugs Chapter I--Food and Drug Administration Department of Health and Human Services. Part 101 Food Labeling. *Reference Amounts Customarily Consumed Per Eating Occasion in the General Food Supply.*(21CFR101.12).
2 As powder, prior to being reconstituted.
3 The large range in serving sizes is due to the inclusion of foods such as imitation bacon bits and bacon strips as well as vegetarian burgers, hotdogs, etc.

The estimated mean consumption of the product for the U.S. population for eaters only (EO) is 10.5 mg/day and 22.4 mg/day for the 90th percentile. On a per capita basis, the estimated consumption is 9.3 mg/day and 21.3 mg/day for the 90th percentile.

D. Basis for GRAS Determination

Through the use of scientific procedures, as described under 21 CFR §170.30(b), Ajinomoto USA (AJ) has determined that dihydrocapsiate is GRAS for its intended uses and is, thus, exempt from the premarket approval requirements of the Federal Food Drug and Cosmetic Act (FFDCA).

The AJ product contains ≥94% of the active chemical moiety (dihydrocapsiate); it is found in a wide variety of chili peppers. Chili peppers are consumed throughout the world for their flavor and pungent heat effects. The concentration of dihydrocapsiate in various chili pepper varietals, the consumption of these varietals in various countries, and the consumption of chili peppers by ethnic populations within countries makes the determination of mean daily consumption of dihydrocapsiate in any particular country (i.e., U.S.) difficult. A dietary survey conducted in 20 populations showed that the intake of chili peppers varied across cultures and even within the same culture, as the mean intake of chili in 13 populations ranged from 0.06% (for Panama Indians) to 3.7% (for Mexican-Americans) of their total diet (Hodun 1980). Thus, while it is clear that dihydrocapsiate is and has been consumed in the U.S. for many years through the practice of eating chili peppers, the quantification of this consumption is difficult and the results unlikely to be reliable.

Metabolic and pharmacokinetic studies indicate that orally-consumed dihydrocapsiate is hydrolyzed rapidly in the GI tract prior to absorption. This rapid metabolism is probably related to the fact that dihydrocapsiate contains an ester linkage rather than the amide linkage as found in capsaicin.

The lability of the ester linkage is the likely explanation for the inability to detect dihydrocapsiate in the plasma of rats or humans. Vanillyl alcohol, a metabolite of dihydrocapsiate, is detected in the portal vein of rats, but at significantly reduced levels and only transiently in the systemic plasma of rats when relatively high doses of dihydrocapsiate (i.e., 19.5 mg/kg) are administered orally.

This suggests that the metabolite (vanillyl alcohol) is subjected to further metabolism, resulting in a sulfate and, to a lesser extent a glucuronide, during its passage through the liver before entering in the systemic blood. After oral administration of ^{14}C-dihydrocapsiate in rats, the plasma $t_{1/2}$ of the radioactivity was 2.4 hr. and excretion of radioactivity, up to 72 hr. after dosing, was 78.2%, 19.4% and 0.5% in the urine, feces and expired air, respectively. Evidence indicates the absence of accumulation in the plasma or the tissues rather, rapid excretion.

As the manufacturing process of the AJ product progressed from "laboratory scale" to "plant scale," there were two changes in the production process of the product precursors (i.e., one change each for MNA and V-OH). Specifically, the use of copper chloride was replaced by copper bromide in the production of MNA and the use of methanol was replaced by tetrahydrofuran, as a solvent, in the production of V-OH. The data available for evaluation in this GRAS determination include results from testing of AJ product from both methods (i.e., laboratory and plant scale dihydrocapsiate); each test identified the method employed to produce the AJ product employed in the toxicological test.

The LD_{50} in mice is >5 g/kg body weight indicating a low level of oral toxicity. Two subchronic (13-week) gavage toxicity studies in rats were performed; both employed daily doses of 100, 300 and 1,000 mg/kg and a concurrent control (0 mg/kg) of medium chain triglyceride. The tests were performed on laboratory and plant scale dihydrocapsiate.

A single test article-related finding was noted by the authors of one study (i.e., hepatocellular hypertrophy in high dose males), whereas the other study was completely without test article-related findings. Thus, the NOAELs for the males were 300 and 1,000 mg/kg from the two studies while the NOAELs for the females were 1,000 mg/kg for both studies. A chronic (26-week) gavage toxicity study in rats was performed; daily doses of 100, 300 and 1,000 mg/kg and a concurrent control (0 mg/kg) of medium chain triglyceride were employed. The NOAEL for male and female rats was 1,000 mg/kg/day. The GRAS Panel reviewed the data from the two 13-week (subchronic) studies and 26-week (chronic) study, and determined that the infrequent occurrence of slight hepatocellular hypertrophy observed in males of one (of the two) 13-week studies was not a toxicological effect.

Teratology studies in rats and rabbits employing doses of 0, 100, 300 and 1,000 mg/kg concluded the NOAEL for general toxicity and reproductive function of the dams and embryo-fetal development was 1,000 mg/kg/day. *In vitro* genotoxicity assays resulted in more negative than positive findings while *in vivo* genotoxicity tests resulted in no positive findings.

Additional supportive safety data are available in the form of studies performed on CH-19 Sweet extract. These data are included for several reasons. First, CH-19 Sweet extract contains approximately 7.5% capsinoids. Second, CH-19 Sweet extract contains approximately 1.5% dihydrocapsiate itself, and third, as is known to the FDA, CH-19 Sweet extract is currently being consumed in the U.S. as a dietary supplement (www.capsiatenatura.com/fda_letter.aspx). The CH-19 Sweet extract data set includes: acute rat toxicity, subchronic 13-week rat toxicity, chronic 26-week rat toxicity, two-generation rat reproduction, rat teratology, rabbit embryo-fetal development, and genotoxicity.

Results from studies in human volunteers are consistent with the findings reported for animals. Oral ingestion of 30 mg of capsinoids, containing 7.92 mg

of dihydrocapsiate, demonstrated no observable pharmacological or toxicological effect, and no detectable plasma concentrations of dihydrocapsiate, or vanillyl alcohol. Plasma concentrations and urinary excretion of catecholamines and their metabolites did not increase (with the exception of MHPG, which was not statistically-significant).

An Expert GRAS Panel (the Panel), the membership of which was chosen by SRA International, Inc. and remained unknown to Ajinomoto USA until after the Panel had met, made its decision, and had issued a written report. The Panel evaluated the GRAS status of dihydrocapsiate for its intended use (i.e., as a direct additive to twenty-eight broad food categories) through the use of scientific procedures, as described under 21 CFR §170.30(b).

The Panel determined that dihydrocapsiate was GRAS for its intended use and thus, exempt from the premarket approval requirements of the Federal Food Drug and Cosmetic Act (FFDCA). Members of the Panel included Samuel M. Cohen, M.D. Ph.D., Alex Makriyannis, Ph.D., Philip S. Portoghese, Ph.D., William J. Waddell, M.D., Bernard M. Wagner, M.D., and Bruce K. Bernard, Ph.D. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients and are well-known to the FDA for their long-standing memberships on GRAS panels. The determinations of this Panel were based upon publically available and accepted information. The data were reviewed and the panel members individually and collectively concluded that there were no data that provided reasonable scientific grounds to suspect, much less demonstrate, a hazard to the public when dihydrocapsiate is used under its conditions of intended use. It was the Panel's opinion that any panel of qualified experts, reviewing the same materials would, based upon scientific procedures, inevitably come to the same conclusion that they did (i.e., that dihydrocapsiate is GRAS under conditions of its intended use in food). See Appendix A for a copy of the GRAS certificate.

E. Availability of Information

The data and information that serve the basis for the GRAS determination request will be sent to the FDA upon request, or are available for FDA's review and copying at reasonable times at the office of SRA International, Inc., 5235 Ragged Point Road, Cambridge, MD 21613, telephone 410-228-1400 and email bernard@sra-intl.com.

2 Identity of the Substance

A. Common or Usual Name

DIHYDROCAPSIATE

B. Chemical Name of the Active Ingredient

Formal Name

(4-hydroxy-3-methoxybenzyl) 8-methylnonanoate

Synonyms

none

The product which is the subject of GRAS determination is dihydrocapsiate prepared by AJINOMOTO. The product is greater than 94% pure and the active chemical moiety (dihydrocapsiate) is naturally-occurring in a wide variety of edible non-pungent as well as pungent chili peppers. The proper uses of dihydrocapsiate are made according to circumstances in this document.

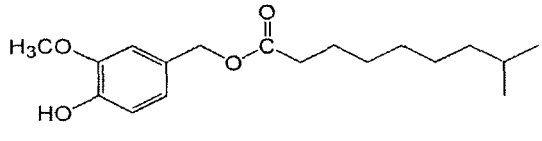
C. CAS Number of the Active Ingredient

205687-03-2

D. Empirical Formula of the Active Ingredient

$C_{18}H_{28}O_4$

E. Structural Formula of the Active Ingredient



F. Molecular Weight of the Active Ingredient

308.41

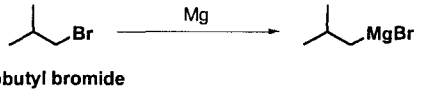
G. Manufacturing

Dihydrocapsiate is a viscous, colorless to yellowish oil. The manufacture of the AJ product starts with the esterification of vanillyl alcohol (V-OH) and 8-methylnonanoic acid (MNA) using an immobilized lipase preparation. The esterification is conducted with N_2 flow under reduced pressure to remove the water.

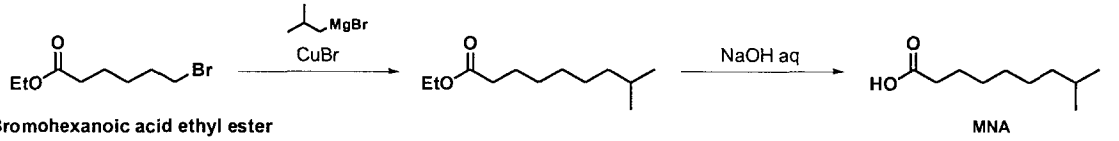
Following the esterification, filtration, extraction with *n*-hexane and evaporation are conducted. The amount of MNA used in the reaction is 1.06 eq. vs. V-OH. The remaining MNA stabilizes dihydrocapsiate by preventing it from de-esterification.

1. Manufacturing Process for MNA

MNA is prepared from isobutyl bromide and 6-bromohexanoic acid ethyl ester through Grignard coupling reaction and deprotection process to give a carboxylic acid.



Isobutyl bromide

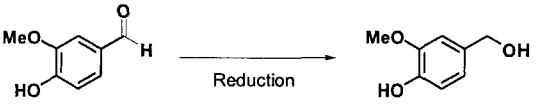


5-Bromohexanoic acid ethyl ester

MNA

2. Manufacturing Process for V-OH

V-OH is prepared from vanillin by reduction. Following reduction, evaporation is conducted.



Vanillin

V-OH

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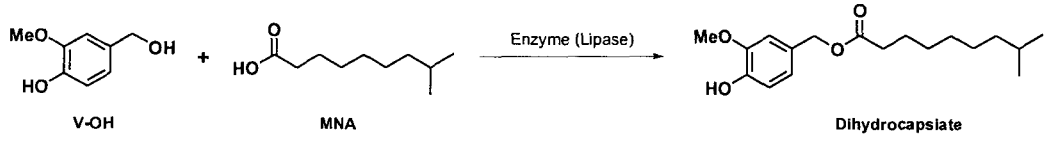
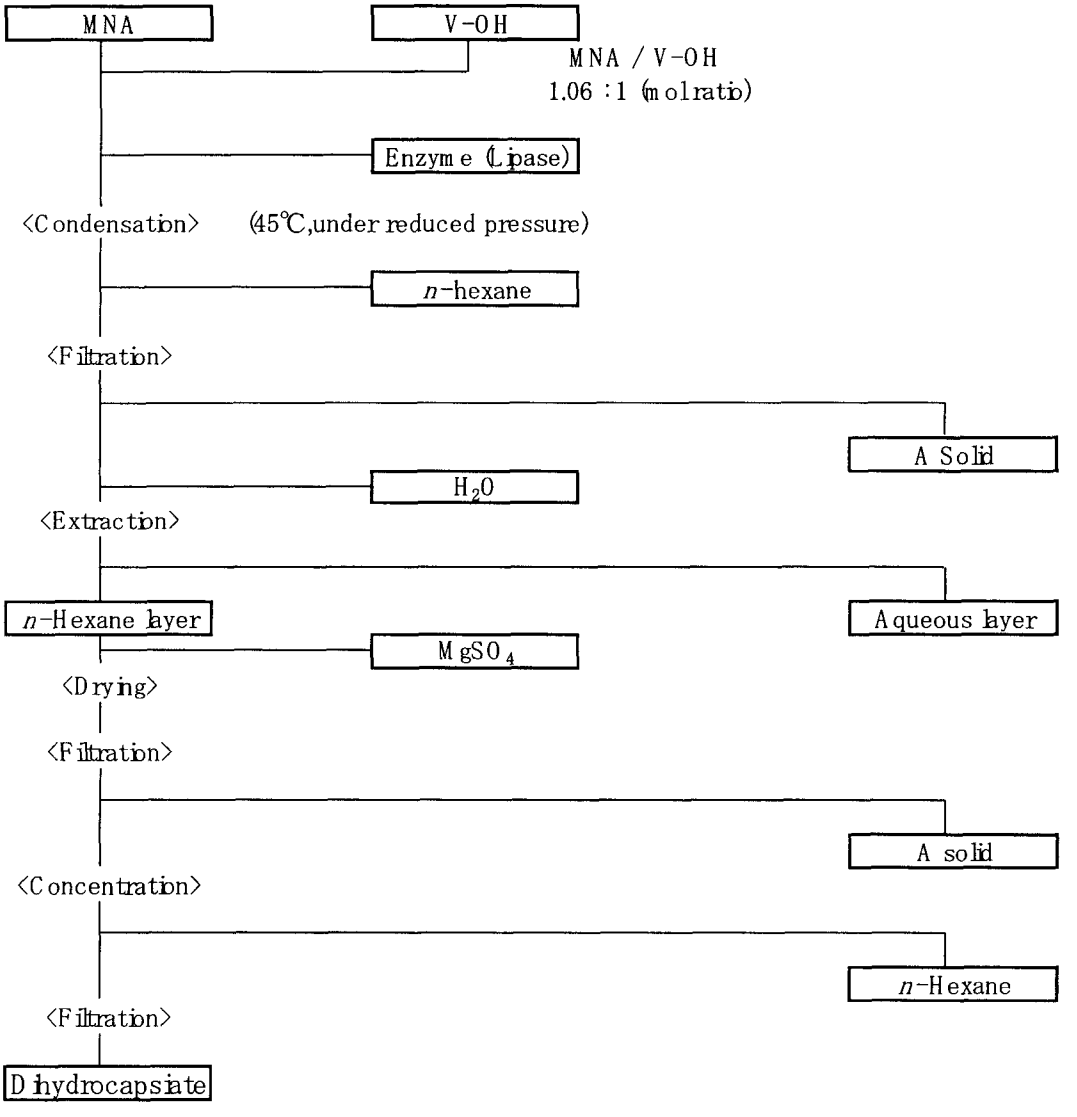
3. Chemical Reaction (Lipase-Catalyzed Esterification)



4. Flow Sheet and Process Control



5. Analytical Results (7 batches)

Table 2. Analytical Results of Actual Production Batches

Test item		Lot No.						
		060626	060705	060712	060720	060731	060807	060817
Description		Viscous, colorless liquid	Viscous, colorless liquid	Viscous, colorless liquid	Viscous, colorless liquid	Viscous, colorless liquid	Viscous, colorless liquid	Viscous, colorless liquid
Identification (IR)		Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms
Specific gravity		1.030	1.030	1.028	1.028	1.024	1.026	1.025
Lead (mg/kg)		<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
Side chain fatty acid Content (%)		2.0	2.0	3.3	3.4	5.8	4.3	4.8
Assay (Dihydrocapsiate) (%)		95.0	95.7	94.2	94.1	93.5	94.0	94.6
Magnesium (mg/kg)		0.3	<0.2	<0.2	<0.2	<0.2	<0.2	0.2
Copper (mg/kg)		<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
Arsenic (mg/kg)		<1	<1	<1	<1	<1	<1	<1
Cadmium (mg/kg)		<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
Residual Solvent (*n*-hexane and tetrahydrofuran [THF], each) (mg/kg)		<5	<5	<5	<5	<5	<5	<5
Related Substances	**Vanillyl Alcohol (%)**	0.04	0.03	0.03	0.03	<0.025	<0.025	<0.025
	Others (%)	1.10	0.86	0.74	0.69	0.81	1.39	0.75

Note : Conforms: It exhibited absorption at the wave number of around 2953cm-1, 2928cm-1, 2855cm-1, 1733cm-1, 1519cm-1, 1278cm-1, 1241cm-1, 1036cm-1, 818cm-1 and 798cm-1.
Quantification Limit: Lead: 0.2 mg/kg, Residual Solvents (*n*-hexane and THF, each): 5 mg/kg, Vanillyl alcohol: 0.025%, Magnesium: 0.2 mg/kg, Copper: 0.2 mg/kg, Arsenic: 0.5 mg/kg, Cadmium: 0.2mg/kg

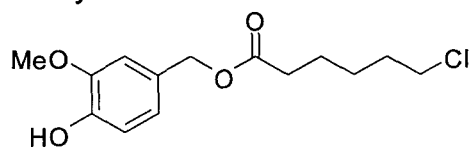
6. Analysis of Other Related Substances

The total percent of other related substances in the seven production batches varied from 0.69% to 1.39%. An analysis was carried out to identify and quantify these substances. The chemical structures for several of the peaks (which accounts for 77% to 91% of total other related substances) were identified (see Figure 1). The identified chemical compounds were evaluated by the GRAS panel and determined not to be of toxicological concern.

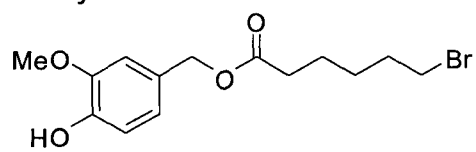
In the seven production batches evaluated by the GRAS Panel, the total unidentified impurity concentration level observed was 0.09 – 0.25%. Using a worst case scenario, wherein a single unidentified contaminant accounts for all of the remaining material (0.25 %) and assuming the mean consumption level for Eaters Only (10.5 mg/day) results in *de minimus* dietary consumption (8.75 ppb).

Figure 1 Five Chemical Structures Identified as Other Related Substances

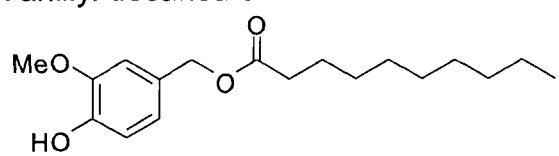
1) Vanillyl 6-chlorohexanoate



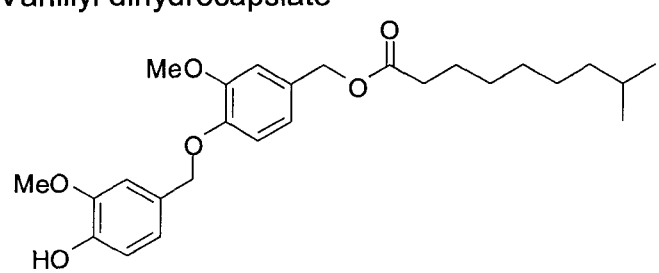
2) Vanillyl 6-bromohexanoate



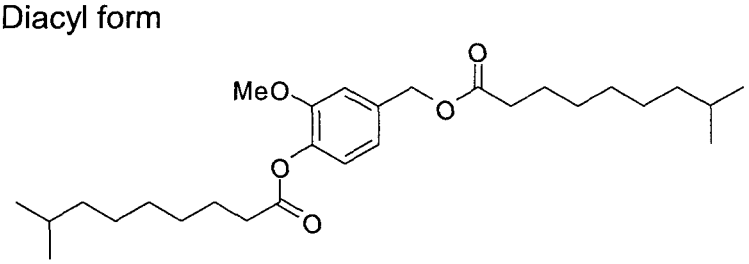
3) Vanillyl decanoate



4) Vanillyl dihydrocapsiate



5) Diacyl form



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H. Specifications for Food Grade Materials

Table 3. Specifications

Test items	Outline of Test Method	Acceptance criteria
Description	JSFA VII, General Notices	Viscous, colorless to yellow liquid
Identification (IR)	FCC V, Infrared Spectra	It exhibits absorption at the wave number of around 2953cm^{-1}, 2928cm^{-1}, 2855cm^{-1}, 1733cm^{-1}, 1519cm^{-1}, 1278cm^{-1}, 1241cm^{-1}, 1036cm^{-1}, 818cm^{-1} and 798cm^{-1}.
Specific Gravity	FCC V, Specific Gravity	1.02 to 1.03
Lead	FCC V, Lead Limit Test, Flame Atomic Absorption Spectrophotometric Method	Not more than 1 mg/kg
Related Substances	HPLC method	Vanillyl alcohol: Not more than 1.0% Total of Other Related Substances : Not more than 2.0%
Residual Solvents (n-Hexane, tetrahydrofuran [THF])	GC method	Not more than 5mg/kg, each
Side chain fatty acid (MNA)	HPLC method	2% to 7%
Assay	HPLC method	≥94%
Magnesium	JSFA VII, Atomic Absorption Spectrophotometry	Not more than 1 mg/kg
Copper	JSFA VII, Atomic Absorption Spectrophotometry	Not more than 1 mg/kg
Arsenic	JP XIV, Arsenic Limit Test, Method 4	Not more than 1 mg/kg
Cadmium	FCC V, Flame Atomic Absorption Spectrophotometric Method	Not more than 1 mg/kg

FCC V: Food Chemicals Codex Fifth Edition
JSFA VII: The Japan's Specifications and Standards for Food Additives Seventh Edition
JP XIV: The Japanese Pharmacopoeia Fourteenth Edition
GC: Gas Chromatography
HPLC: High-Performance Liquid Chromatography

3 Intended Use and Consumer Exposure

A History of Use

1. Natural Presence of Dihydrocapsiate in "Chili Peppers"

Dihydrocapsiate and capsiate were first discovered in CH-19 Sweet, a nonpungent chili pepper (Yazawa et al. 1989). Dihydrocapsiate (Figure 2) and capsiate (Figure 3) are analogues of capsaicin (Figure 4), which have an ester bond in place of the amide bond between the vanillyl moiety and fatty acid moiety compared to capsaicin (Kobata et al. 1998). Kobata et al. (1999) also isolated a novel capsaicinoid-like substance from CH-19 Sweet, and named it nordihydrocapsiate (Figure 5). As a group, dihydrocapsiate, capsiate and nordihydrocapsiate are known as capsinoids.

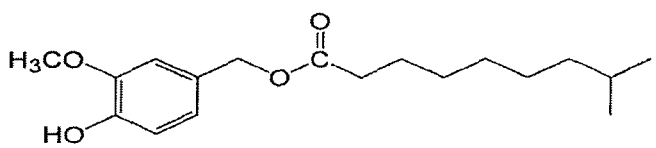

Figure 2. Chemical structure of dihydrocapsiate

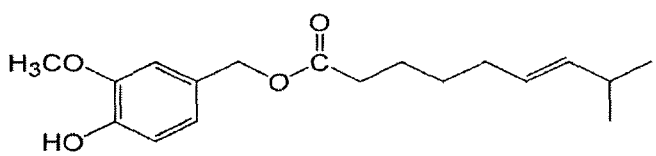

Figure 3. Chemical structure of capsiate

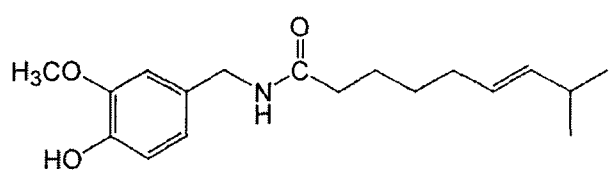

Figure 4. Chemical structure of capsaicin

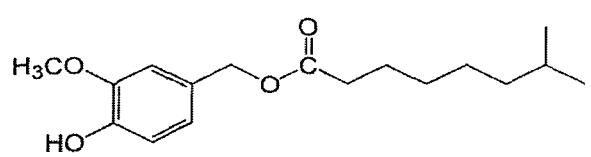

Figure 5. Chemical structure of nordihydrocapsiate

Capsaicinoids in chili peppers are biosynthesized from branched chain amino acids (BCAAs) and phenylalanine in the pepper fruits. BCAAs are converted to the side-chain fatty acid, and phenylalanine is converted to vanillylamine or vanillyl alcohol via vanillin. Finally, capsaicinoids are biosynthesized from the side-chain fatty acid and vanillylamine. On the other hand, capsinoids, including dihydrocapsiate, are biosynthesized from the side chain fatty acid and vanillyl alcohol. Recently, Sutoh et al. (2006) reported that vanillylamine, a precursor of capsaicin, was biosynthesized in pungent peppers, but not in CH-19 Sweet, whereas vanillyl alcohol was biosynthesized in both pungent peppers and CH-19 Sweet. These results suggest that dihydrocapsiate and other capsinoids are biosynthesized not only in CH-19 Sweet, but also in pungent peppers.

Recently, researchers at the University of Maine and USDA(*1) demonstrated the occurrence of dihydrocapsiate in several kinds of chili peppers (*Capsicum* fruits) in the USDA's *Capsicum* germplasm collection, and published the results (Table 4) at the IFT (International Food Technology) annual meeting 2007 (Jul 28th-Aug 1th, 2007, Chicago, U.S.A.).

Table 4. Quantified amount of Dihydrocapsiate in pepper samples

Pepper sample	Dihydrocapsiate (µg/g)
PB26	96.5377*
PE56	81.4507 (+ / - 1.0473)
NP30	55.4476 (+ / - 0.1053)
NP42	20.0454 (+ / - 1.2824)
PE86	37.4338 (+ / - 1.3338)
PE31	23.7578*
NP37	38.6705 (+ / - 1.302)
441680	13.0296 (+ / - 0.7698)
PE66	39.4183 (+ / - 0.9656)
PB35	50.6139 (+ / - 0.8725)

*indicates insufficient sample to conduct a triplicate analysis

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Furthermore, Yazawa et al. (2004) reported on the amount of capsinoids (including, but not limited to dihydrocapsiate) found in various edible chili pepper cultivars (Table 5).

The above results confirm that dihydrocapsiate is naturally-occurring in various edible chili peppers.

Table 5. Capsinoids content in various chili peppers

Variety (Cultivar)			Capsinoids(mg/g DW)
CH-19 Sweet (AMA)	*C. annuum*	Japan	1.818
Nikko	*C. annuum*	Japan	0.159
Cone pepper	*C. annuum*	Japan	0.155
Tsumura	*C. annuum*	Japan	0.04
Yokaku	*C. annuum*	Japan	0.051
Goshiki	*C. annuum*	Japan	0.131
Cherry pepper	*C. annuum*	Japan	0.073
Cayenne long slim	*C. annuum*	USA	0.079
Hungarian yellow wax	*C. annuum*	USA	0.009
Habanero	*C. chinense*	Mexico	0.037
Af-8	*C. baccatum*	Ivory Coast	0.052
Red cluster pepper	*C. annuum*	Japan	0.082
Aroma-Af3	*C. chinense*	Rwanda	1.106
Sy-2	*C. chinense*	Seychelles	0.71

2 History of use for "Chili Peppers," *Capsicum annuum* L

Capsicum annuum L. is an herbaceous annual that reaches a height of one meter and has lanceolate leaves, white flowers, and fruit that vary in length, color (from green to red to purple) and pungency, depending upon the cultivar. The fruit contains many flat, kidney-shaped, white seeds that may be pungent tasting.

The terms "*capsicum* peppers" refer primarily to the *Capsicum annuum* L. species plants used in the manufacture of selected commercial products known for their strong pungency and/or color. *Capsicum* peppers, known for their pungency (i.e., capsaicinoid concentration), are referred to by consumers more generically as "hot peppers" or "chili peppers."

Chili peppers have been a part of the human diet in South, Middle, and North America since approximately 7500 BC. The plants were domesticated between 5200 and 3400 BC, constituting one of the first cultivated crops in the Americas (Wikipedia Encyclopedia). Christopher Columbus was one of the first Europeans to encounter chili peppers, and called them 'peppers' because of their similarity in taste (though not in appearance) with the Old World peppers of the Piper genus (i.e., Piper nigrum, the source of black pepper). Diego Álvarez Chanca, a physician on Columbus' second voyage to the West Indies in 1493, brought the first chili peppers to Spain and first wrote about their medicinal effects in 1494. Chili peppers spread rapidly from Mexico into the Philippines, then to India, China, and Japan with the aid of European sailors. The new spice was quickly incorporated into local cuisines (AMPI 1997).

Various *Capsicum* species are used fresh or dried, whole or ground, and alone or in combination with other flavoring agents. *Capsicum annuum* L. is used in paprika, pimento, and other red pepper products, with paprika used primarily in the flavoring of garnishes, pickles, meats, barbecue sauces, ketchup, cheese, snack food, dips, chili con carne, salads, and sausages. Spanish paprika is called pimento and is generally used for coloring purposes. Chilies and chili peppers from cultivars of *Capsicum annuum* L. are used as flavoring in many foods, such as curry powder and Tabasco® sauce. *Cayenne* pepper is the ground product derived from the smaller, most pungent *Capsicum* species.

As a medicinal plant, the *Capsicum* species has been used as a carminative, digestive irritant, stomachic, stimulant, rubefacient, and tonic. The plants have also been used as folk remedies for dropsy, colic, diarrhea, asthma, arthritis, muscle cramps, and toothache (Govindarajan and Sathyanarayana 1991).

3 Dietary Consumption of Dihydrocapsiate from "Chili Peppers," *Capsicum annuum* L

Chili peppers are consumed throughout the world and are appreciated for their flavor and pungent heat effects. The amount used varies from country-to-country. For example, it was reported that the mean daily consumption of chili peppers in Mexico, Korea, Thailand, India and the United States are 15, 8, 5, 2.5, and 0.05-0.5 g/person/day, respectively (Govindarajan and Sathyanarayana 1991).

A dietary survey conducted of 20 populations showed that the intake of chili peppers varied across cultures and even within the same cultures, as the mean intake of chili in 13 populations ranged from 0.06% (for Panama Indians) to 3.7% (for Mexican-Americans) of their total diet (Hodun 1980).

Considering that the amount of dihydrocapsiate in chili peppers varies according to the variety of chili peppers, it is difficult to estimate the consumed amount of dihydrocapsiate precisely. However, it is clear through the custom of eating chili peppers that people in the United States commonly consume dihydrocapsiate from them.

4 Regulatory

The Food and Drug Administration issued a no-objection-letter (Tarantino to Bernard, March 9, 2009) in response to GRAS Notice No. GRN 000249, dated April 17, 2008 submitted by SRA International on behalf of Ajinomoto USA, Inc. The notice informed FDA that the use of synthetic dihydrocapsiate was GRAS, through scientific procedures, for use a food ingredient in a variety of food categories, as presented below in Table 6, to provide one milligram synthetic dihydrocapsiate per standard serving[2].

[2] Reference amounts customarily consumed (21 CFR 101.12)

Table 6 Food Categories and Intended Use Levels included in GRN No. 000249

Food Group	Mg Dihydrocapsiate/ Serving Size	Use Level (ppm)
Meal replacement and yogurt beverages – low-calorie	1.0	4.2
Tea sugar-free	1.0	4.2
Fruit juice – fresh orange juice	1.0	4.2
Vegetable juice	1.0	4.2
Fruit-aides, drinks, and powders – low-calorie	1.0	4.2
Beverage concentrates - low calorie	1.0	4.2
Beverage soft drinks – sugar-free	1.0	4.2
Non-carbonated water – low-calorie	1.0	4.2
Energy drinks	1.0	4.2
Yogurt – Fruit – Non and low Fat	1.0	4.4
Yogurt – Chocolate, Vanilla, Plain – Non and low fat	1.0	4.4
Oatmeal – instant, low-sugar	1.0	4.2
Chewing gum – sugarless	1.0	333
Mints dietetic, low-calorie	1.0	500
Breakfast and meal replacement bars	1.0	25
Gelatin/Puddings – low-calorie	1.0	8.3
Salad dressings – low-calorie	1.0	33
Sweeteners – low-calorie	1.0	250
Snack foods – popped-low-calorie – low fat and rice cakes	1.0	33
Frozen Desserts – light	1.0	
Frozen desserts - ice pops and fruit bars	1.0	11.8
Frozen desserts – dairy	1.0	8.3
Ready to eat meals	1.0	
Ready to eat meals – frozen diet (non-meat, non-chicken and non-fish/seafood)	1.0	4.2
Ready to eat meals – soup	1.0	4.1
Nutritional meal – Ensure	1.0	4.2

CH-19 Sweet extract is a mixture of capsinoids consisting of dihydrocapsiate, capsiate and nordihydrocapsiate present in the refined and concentrated oil extracted from CH-19 Sweet, a non-pungent cultivar of *Capsicum annuum* L. With regard to CH-19 Sweet extract, a New Dietary Ingredient Notification was submitted to FDA by the applicant. The notification was filed by the FDA without any objection on May 17, 2007 (http://www.capsiatenatura.com/fda_letter.aspx)

B. Intended Use and Consumption Estimates

The applicant intends to enrich foods with dihydrocapsiate at a use level of 3 mg per standard serving (except in chewing gum) as a source of dihydrocapsiate in the diet. The applicant retained Exponent, Inc. (Washington, DC), to estimate the dietary intake from selected foods enriched with dihydrocapsiate for the total US population and the following subpopulation groups: teenagers 13-19, adults 20-39 years, 40-55 years and adults 56+ years.

Dietary intake of dihydrocapsiate was estimated using consumption data from the 2003-06 National Health and Nutrition Examination Survey (NHANES) and Exponent's Foods Analysis and Residue Evaluation Program (FARE™) software version 8.04. The intakes are based on 2-day average daily intakes among subjects with 2 days of valid data. Both per capita and per user intakes were estimated.

1. Food Categories and Use Levels

The proposed food categories included in the analysis and their associated use levels are presented in Table 7.

Table 7. Proposed uses and use levels of capsiate in various light and low calorie foods

Food Group	Mg Dihydrocapsiate per Serving Size	FDA 21 CFR 101.12 Serving Size (g)[1]	Use Level (ppm)
Bars - Breakfast and meal replacement bars	3.0	40	75
Beverage concentrates[2] – low-calorie	3.0	21.6	138.9
Beverages soft drinks – sugar free	3.0	240	12.5
Chewing gun – sugarless	10.0	3	3333.3
Cookies – low fat and non-fat	3.0	30	100
Creamer	3.0	15 - 32	93.8 – 200
Energy drinks	3.0	240	12.5
Frozen Desserts – light	3.0		
Frozen desserts - ice pops and fruit bars	3.0	85	35.3
Frozen desserts – dairy	3.0	120 - 240	12.5 – 33.3
Fruit-aides, drinks, and powders – low calorie	3.0	240	12.5
Fruit juice – fresh orange juice	3.0	240	12.5
Gelatin/Puddings – low-calorie	3.0	120 - 147	20.4 – 25
Hard candy, dietetic (previously limited to mints)	3.0	2 - 40	75 -1500
Liquid coffee	3.0	240	12.5
Low-fat and non-fat crackers	3.0	30	100
Meal replacement beverages	3.0	30[3] - 240	12.5 - 100
Non-carbonated water – low calorie	3.0	240	12.5
Nutritional meal - Ensure	3.0	240	12.5
Oatmeal – instant, low sugar	3.0	240	12.5
Protein based meat alternative	3.0	7 - 240[3]	12.5 - 428.6
Ready to eat meals	3.0		
Ready to eat meals – frozen diet (non meat, non-chicken, non fish/seafood)	3.0	240	12.5
Ready to eat meals – soup	3.0	245	12.2
Ready to eat cereals, cold	3.0	15 - 55	54.5 - 200
Salad dressings – low-calorie	3.0	15 - 30	100 - 200
Snack foods – popped-low-calorie – low fat and rice cakes	3.0	30	100
Sweeteners – low-calorie	3.0	4	750
Tea, liquid low sugar or sugar fee	3.0	240	12.5
Vegetable juice	3.0	240	12.5
Yogurt – Fruit – Non and low Fat	3.0	225	13.3
Yogurt – Chocolate, Vanilla, Plain – Non and low fat	3.0	225	13.3

1 Title 21--Food and Drugs Chapter I--Food and Drug Administration Department of Health and Human Services. Part 101 Food Labeling. *Reference Amounts Customarily Consumed Per Eating Occasion in the General Food Supply.*(21CFR101.12).
2 As powder, prior to being reconstituted.
3 The large range in serving sizes is due to the inclusion of foods such as imitation bacon bits and bacon strips as well as vegetarian burgers, hotdogs, etc.

2. Estimated Consumption

The estimated mean consumption for the US population for eaters only (EO) is 10.5 mg/day and 22.4 mg/day for the 90th percentile[3]. On a per capita basis, the estimated consumption is 9.3 mg/day and 21.3 mg/day for the 90th percentile.

The food category with the highest estimated daily intake of dihydrocapsiate was liquid coffee with a mean per user intake of 6.9 mg/day among the total US population; the mean 90th percentile per user intake is 13.4 mg/day.

Mean estimated daily intake per user for teenagers (13-19 years) was 6.4 mg/day and 12.7 mg/day in the 90th percentile. The category with the highest intake was among consumers of fruit drinks (11.4 mg/day, 90th percentile) and non-carbonated water consumers (14.8 mg/kg/day, 90th percentile).

The highest estimated daily intake for adults was among consumers of sugar-free soft drinks. The 90th percentile estimated consumption for adults 20-39 years was 16.0 mg/day, while the mean intake from all enriched foods was estimated to 10.2 mg/day and 21.6 mg/day for the 90th percentile. The highest intake was seen among consumers of non-carbonated water, however the sample size (N=21) was too small to be statistically reliable. The highest estimated daily intake for adults 40-55 years old and 56 years and older was also among consumers of sugar-free soft drinks (17.0 mg/day and 13.5 mg/day at the 90th percentile). Total intake for adults 40-55 years old was estimated to be 14.0 mg/day (mean) and 27.8 mg/day (90th percentile). Adults 56 years and older had an estimated mean total intake of 12.9 mg/day and 24.1 mg/day at the 90th percentile.

[3] Due to small sample sizes, few of the 90th percentile results can be estimated with statistical reliability. A sample size of 160 is necessary to reliably estimate the 90th percentile from a population distribution with an estimated design effect of 2.0, as recommended by NCHS[2]. There were no consumers in the NHANES 2003-04 survey who reported drinking beverage concentrated, low calorie. http://www.cdc.doc/nchs/data/nhanes/nhanes3/nh3gui.pdf

4 Toxicological Studies

A. Change in Production Process

In 2006, due to progression from "laboratory scale" production to "plant scale" production, there were two changes in the production process of the dihydrocapsiate precursors (i.e., MNA and V-OH).

As shown on pages 10 – 12, the manufacture of dihydrocapsiate is initiated with the combining of the basic building blocks of the reaction (i.e., MNA and V-OH) with an enzyme (lipase).

1. Specific Changes in Production of MNA and V-OH

MNA Production: Change from Copper Chloride to Copper Bromide
The manufacture of MNA for the current "plant scale" production process is shown in Section G (Manufacturing),1 on page 10. It employs copper bromide in the 'coupling reaction' whereas the initial "laboratory scale" production process employed copper chloride at this step.

V-OH Production: Change from Methanol (as a solvent) to Tetrahydrofuran (THF)
For the current "plant scale" production process of V-OH, as shown in Section G, (Manufacturing), 2 on page 10, tetrahydrofuran (THF) is employed as the reaction solvent. In the initial "laboratory scale" production process, methanol was employed as the reaction solvent.

2. Production Process and Toxicological Data

Dihydrocapsiate prepared using either "laboratory scale" or "plant scale" processes was employed in standardized toxicology studies. The following is a listing of studies performed together with the production process ("laboratory scale" or "plant scale") used to produce the material.

Table 8. Toxicological Studies

Study Name	Process	Lot#
Limit Test (Acute Toxicity) in Mice	Plant scale	060807
A 13-Week Oral Toxicity Study in Rats	Laboratory scale	WKU05137ZBa
A 13-Week Oral Toxicity Study in Rats	Plant scale	060807
Bacterial Reverse Mutation Test	Laboratory scale	WKU05137ZBa
Chromosome Aberration Test in Cultured Mammalian Cells	Laboratory scale	WKU05137ZBa
Micronucleus Test in Mice	Laboratory scale	WKU05137ZBa
Micronucleus Test in Mice	Plant scale	060807
Single Cell Gel (SCG) Assay with Rats- Comet Assay	Laboratory scale	WKU05137ZBa
Gene Mutation Assay in Transgenic Rats	Plant scale	060807
Teratological Study In Rats	Plant scale	060807
Teratological Study In Rabbits	Plant scale	060807
A 26-week Oral Toxicity Study in Rats	Plant scale	070813

3. Comparison of Analytical Data by Process

The comparison of the analytical data of the dihydrocapsiate samples between the two processes ("laboratory scale" and "plant scale") are presented in Table 9 below.

Table 9. Analytical Data by Process

Test item		Lot No.		
		Laboratory Scale Process	Plant Scale Process	
		WKU05137ZBa	**060807**	**070813**
Description		Viscous, pale yellow liquid	Viscous, colorless liquid	Viscous, colorless liquid
Identification (IR)		Conforms	Conforms	Conforms
Specific gravity		-	1.026	1.026
Lead (mg/kg)		-	<0.2	<0.2
Side chain fatty acid Content (%)		2.7	4.3	3.9
Assay (Dihydrocapsiate) (%)		95.8	94.0	95.4
Magnesium (mg/kg)		-	<0.2	<0.2
Copper (mg/kg)		-	<0.2	<0.2
Arsenic (mg/kg)		-	<1	<1
Residual Solvent (*n*-hexane) (mg/kg)		-	<5	<5
Related Substances	**Vanillyl Alcohol (%)**	0.05	<0.025	0.11
	Others (%)	2.02	1.39	0.55

- : Not Tested

B. Toxicology Studies Performed Using "Plant Scale" Samples

1. Acute Toxicity

Mice, male and female ICR, 5/sex/group, gavage, single dose, 5000 mg/kg, Vehicle: medium chain triglyceride; 14-day observation period.

Findings:
During the observation period of acute toxicity for 2 hours after dosing, staggering gait, decrease in spontaneous movement, prone position, tremor, gasping or red-brownish urine were observed in males or females in the treated group. All of these findings resolved 6 hours post-dosing. These changes were determined to be transient. No deaths were observed.

Conclusion:
This study concluded that the LD50 is greater than 5000 mg/kg. (Watanabe et al. 2008c)

Study Evaluation:
Standard limit test design, dose, and observation period employed.

2. Genotoxicity

Gene Mutation Assay
Rat, male Big Blue™ transgenic: 5/group, gavage; Dose: negative control, positive control (7,12-dimethylbenzo[*a*]anthracene [DMBA]), 500, 1000 mg/kg, 28 days; Vehicle: medium chain triglyceride; Target tissues: liver, kidney, intestinal tract (duodenum).

Body Weight and General Conditions
There were no untoward effects on body weight. No toxicological effects were observed.

Findings: Liver

The negative control demonstrated a mutant frequency of 35.7 x 10^{-6}. The mean among individuals was 36.7 x 10^{-6}. Mutant frequencies of 24.5 x 10^{-6} and 28.1 x 10^{-6} were observed in the low- and high-dose groups respectively. The means among individuals in the low- and high-dose groups were 24.0 x 10^{-6} and 28.2 x 10^{-6}, respectively. The findings in the treatment groups were comparable to the negative control group.

The positive control group exhibited a mutant frequency of 118.3 x 10^{-6}, which was statistically-significant as compared to negative controls. The mean among the individuals was 115.2 x 10^{-6}.

Findings: Kidney

The negative control demonstrated a mutant frequency of 22.5 x 10^{-6}. The mean among individuals was 22.3 x 10^{-6}. Mutant frequencies of 28.7 x 10^{-6} and 30.1 x 10^{-6} was observed in the low- and high-dose groups respectively. The means among individuals in the low- and high-dose groups were 29.7 x 10^{-6} and 30.1 x 10^{-6}, respectively. The findings in the treatment groups were comparable to the negative control group.

The positive control group exhibited a mutant frequency of 75.8 x 10^{-6}, which was statistically-significant as compared to negative controls. The mean among the individuals was 82.7 x 10^{-6}.

Findings: Duodenum

The negative control demonstrated a mutant frequency of 43.5 x 10^{-6}. The mean among individuals was 44.2 x 10^{-6}. Mutant frequencies of 36.8 x 10^{-6} and 32.0 x 10^{-6} were observed in the low- and high-dose groups respectively. The means among individuals in the low- and high-dose groups were 35.7 x 10^{-6} and 32.3 x 10^{-6}, respectively. The findings in the treatment groups were comparable to the negative control group.

The positive control group exhibited a mutant frequency of 237.9 x 10^{-6}, which was statistically-significant as compared to negative controls. The mean individual mutant frequency was 237.5 x 10^{-6}.

Results: Negative. There were no statistically-significant differences between the treatment groups as compared to the negative control group. (Bernard et al. 2008a)

Study Evaluation:
Standard study design employed; used concurrent positive and negative controls. Highest dose referred to the OECD in vivo genotoxicity guideline employed (1,000 mg/kg).

Mouse Micronucleus Test
Test system: mouse, male BDF_1; 5/group.
Design: gavage; dose: negative control, positive control (Mitomycin C), 500, 1000, 2000 mg/kg, 2 days.
Vehicle: medium chain triglyceride

When compared to the negative control group, no statically significant increase in the incidence of micronucleated polychromatic erythrocytes (MNPCE) was observed in treatment group.

The positive control group which received 0.5 mg/kg Mitomycin C intraperitoneally, exhibited a marked increase. This increase was statistically-significant ($p \leq 0.025$) compared to the negative control group.

Results: Negative. (Watanabe et al. 2008c)

Study Evaluation:
Standard study design employed; used concurrent positive and negative controls. Employed highest recommended dose (2000 mg/kg).

3. Subchronic (13-Week Study) Toxicity in Rats

Rat, male and female Sprague-Dawley; 10/sex/group, Gavage; Dose: 0, 100, 300, 1000 mg/kg, Vehicle: medium chain triglyceride.

Findings:
No deaths occurred during the study and no abnormalities attributed to the test article were observed. There were no statistically-significant differences in body weights.

There were increases of food consumption in male high-dose group near the end of the study. These were not always statistically-significant and were determined to be unrelated to the test article. Female food consumption was not statistically-different when compared to controls. There were no test substance-related changes in water intake.

No hematological changes due to test article administration were observed.

Blood chemical analyses revealed a significant increase in ALT activity in the high-dose group in both sexes. Male high-dose total protein was also increased. Males in this group showed a significant decrease in creatine which was determined not to be of toxicological significance.

Males in the high-dose group demonstrated an increase in urine volume and excretion of sodium, potassium and chloride. As these parameters were not significantly altered in the blood; the observations in the urine were determined not to be of toxicological significance.

There were no abnormalities present in ophthalmologic examination.

High-dose males demonstrated a significant increase in absolute and relative liver and kidney weight. High-dose females demonstrated a similar effect in relative liver and kidney weight. However, there was no corresponding histopathological finding. A statistically-significant decrease in absolute and relative organ weight of the thymus were observed in low-dose females. This change was deemed not to be dose-related.

Histopathologic change was observed in the liver of the high-dose males (hypertrophy of the hepatocyte). Other changes were determined to be incidental or spontaneous (frequently observed in this strain of rats).

Conclusion:
The study concluded that hypertrophy of the hepatocyte was a test-article related change in high-dose males. The study authors, in the absence of a chronic study or data demonstrating the reversal of the finding with withdrawal of exposure (i.e., recovery group), concluded the NOAELs were 300 mg/kg/day for males and 1000 mg/kg/day for females. (Watanabe et al. 2008a). This conclusion was modified by the Expert GRAS Panel when data from a chronic study containing recovery groups was generated (see below).

Study Evaluation:
Standard study design employed; compound related effects obtained at the high dose.

4. Chronic (26-Week) Toxicity in Rats

Rat, male and female Sprague-Dawley; 10/sex/group, Gavage; Dose: 0, 100, 300, 1000 mg/kg, Vehicle: medium chain triglyceride.

Findings:
No deaths occurred during the dosing or recovery periods. On Day 127, one high-dose male was sacrificed in a moribund condition (demonstrated prone/lateral position and bradypnea). Subcutaneous masses (neck or axillary region) were observed in two high-dose females. One female was sacrificed at dose-termination. The other female continued to demonstrated subcutaneous masses in the neck was during the recovery period. All other rats appeared normal during the study and observation period. There were no statistically-significant differences in body weights during the 26-week dosing period or the 4-week recovery period.

Increased food consumption as compared to controls was observed in the female high-dose group beginning on Day 77 and continued through the end of the recovery period. Food consumption in all other female groups (low- and mid-dose) and all male groups was comparative to controls.

During test article administration, water consumption was higher in male and female high-dose groups, however the increase was statistically-significant only in females and only on Days 25 and 144. During the recovery periods, water consumption was increased only in high-dose females and only statistically-significant on recovery Day 28.

At the end of test article administration, hematology analyses revealed statistically-significant changes in all treatment groups. The high-dose groups demonstrated statistically-significant mean differences in the following parameters: increased platelet count, decreased neutrophil ratio (males only),

increase in mean corpuscular volume, and decrease in red blood cells (females only). A statistically-significant decrease in fibrinogen was observed in mid-dose males and females. The low-dose group demonstrated a statistically-significant increased number of eosinophils (females only). The only finding that continued through the observation period was the increased platelet count in high-dose males. Females demonstrated no abnormalities during the recovery phase.

At the end of dosing, blood chemistry analyses revealed statistically-significant increases as compared to controls in the high- and mid-dose groups. In the high-dose group statistically-significant increases were observed in males and females in ALT, total protein, and calcium. Males also demonstrated statistically-significant increases in phospholipids, total cholesterol and α_2-globulin fraction. Statistically-significant decreases in high-dose females were noted in γ-globulin fraction and creatinine. In mid-dose males, several blood chemistry parameters achieved statistically-significant increases: ALT activity, phospholipids, and α_2-globulin fraction ($p<0.01$). At the end of the recovery period, two parameters in high-dose rats were statistically-significant: a decrease in β-globulin fraction (male and females), and an increase in α_1-globulin fraction (males only).

At the end of administration, high-dose males and females had a tendency toward decreased number of positive reactions for ketone body due as result of vehicle metabolism. Males in the high-dose group demonstrated statistically-significant increases in urine volume and sodium, potassium and chloride excretion. Similar increases were not observed in females at the end of administration. No differences were observed in males during the recovery period. During the recovery phase, high-dose females demonstrated statistically-significant increases in urine volume, sodium, potassium and chloride excretion. These changes were considered random given the absence of similar changes in serum electrolytes.

Ophthalmological findings were unaffected by administration of the test article.

At the end of test administration, both males and females in the high-dose group demonstrated statistically-significant increases in absolute and relative liver weight. Additional statistically-significant increases observed in high-dose males were in the relative weight of the kidney, heart, spleen, and salivary gland. Statistically-significant changes also observed in high-dose females were increases absolute weight of the kidney, heart, salivary glands, pituitary, and lung. Mid-dose females demonstrated a statistically-significant increase in absolute weight of the pituitary. Following the recovery period, statistically-significant increases in absolute and relative weight of the pituitary were observed in high-dose females, in addition to increases in absolute weight of salivary and adrenal glands, and relative liver weight.

One high-dose male, sacrificed in moribund condition, presented with a cerebral nodule. No test article-related changes were observed in any animal following test article administration or the recovery period.

Oligodendroglioma was observed in the male rat (high-dose group) which had been sacrificed moribund. This corresponded to the nodule observed at necropsy. While there were minimal changes observed in the liver of the high-dose group, these changes were not statistically-significant. In high-dose females, at termination of dosing, statistically-significant increased incidences of kidney tubule regeneration and kidney urinary cyst hyaline were observed. Two adenocarcinomas and increased incidence of mammary gland focal or lobular hyperplasia were observed in high-dose females at the end of the recovery period. These findings and other observations were judged to be incidental or spontaneous for this strain of rat.

Conclusion:
The study concluded that the NOAEL was 1,000 mg/kg for both male and female rats. (Kodama et al. 2009).

Study Evaluation:

Standard study design employed; compound related effects obtained at the high dose and the MOS was high (>6,000x the anticipated human consumption).

5. Teratology (13-Week Study) Toxicity in Rats

Evaluation in the Sprague-Dawley Rat

Rat, pregnant female, 19 to 20/group; gavage; Dosing Day 7 – Day 17 of gestation, Doses: 0, 100, 300, 1000 mg/kg/day; Vehicle: medium chain triglyceride.

Findings: Effects on Dams

There were no deaths in any group. There were no significant differences between the control group and the treated groups with regard to body weight during gestation, or in body weight gain during test article administration (Day 7 to Day 18 of gestation) or following administration (Day 18 to Day 20 of gestation). No significant differences in mean one-day food consumption during the gestation period were observed. Macroscopic examination revealed no abnormalities in any major organs/tissues in the thoracic or abdominal cavity in any group.

Findings: Effects on Embryo/Fetal Development

No significant differences in the number of *corpora lutea*, number of implantations, index of resorbed/dead fetuses, number of live fetuses or sex ratio were observed between the control group and treated groups. The mid-dose group experienced a significant decrease in the index of implantations. The finding was only in the mid-dose group and determined to not be dose-related. Males in the high-dose group demonstrated a significant increase in body weight as compared to the control group. The body weights of the high-dose group

were within the range of background data for the testing facility and was not associated with a delay in fetal development.

There were no gross pathological abnormalities of the placenta observed in any group.

With regard to external abnormalities, rudimentary tails were observed in one fetus each in the control group and mid-dose group. This finding was not dose-dependent. The following visceral abnormalities were noted: tetralogy of Fallot, ventricular septal defect, right ventricular hypertrophy, absence of aortic arch, abnormal origin of right subclavian artery and abnormal lobation of the liver. The number of fetuses/dam/group with visceral abnormalities were 2 fetuses in one dam in the control group, one fetus each in 6 dams in the low-dose group, a total of 4 fetuses in 3 dams in the mid-dose group, and a total of 3 fetuses in 2 dams in the high-dose group. The incidence index of abnormal lobation of the liver was significantly higher in the low-dose group. This finding was limited to the low-dose group and considered not to be dose-dependent. There were no significant differences between the control group and any test article administration group.

Visceral variations observed included thymic remnant in neck, dilatation of renal pelvis, dilatation of ureter, convoluted ureter and left umbilical artery. Variations were observed in a total of 8 fetuses in 5 dams in the control group, in a total of 6 of fetuses in 5 dams in the low-dose group, in a total of 4 fetuses in 3 dams in the mid-dose group, and in 1 fetus each in 2 dams in the high-dose group. There were no significant differences in the incidence index between controls and treated groups.

Skeletal abnormalities observed included fusion of mandibulae, misshapen presphenoid bone, absence of cervical vertebra and wavy ribs. Abnormalities were observed in 1 fetus in 1 dam in the control group, in a total of 5 fetuses in 2 dams in the low-dose group, 2 fetuses in 1 dam in the mid-dose group, and 2

fetuses in 1 dam in the high-dose group.

There were no significant differences in the incidence index between controls and treated groups.

Skeletal variations observed included cervical rib, shortened 13th rib, 14th rib, splitting of thoracic vertebral body, asymmetry of sternebra, and lumbarization of sacral vertebra. Skeletal variations were observed in a total of 27 fetuses in 12 dams in the control group, in a total of 30 fetuses in 14 dams in the low-dose group, in a total of 34 fetuses in 13 dams in the mid-dose group, and a total of 31 fetuses in 16 dams in the high-dose group. There were no significant differences in the incidence index between controls and treated groups.

With regard to progress of ossification, there were no significant differences in the number of ossified metacarpi, ossified metatarsi, ossified sacral and caudal vertebrae, or in the index of ossified sternebrae between the control group and any test substance administration group.

Conclusion:
The study concluded the NOAEL for general toxicity on dams, reproductive functions of dams and embryo-fetal development was 1000 mg/kg/day. (Bernard et al. 2008b)

Study Evaluation:
Standard study design employed; no toxicity was observed.

Evaluation in the New Zealand White Rabbit

Rabbit, pregnant female, 19 to 21/group; gavage; Dose: 0, 100, 300, 1000 mg/kg/day,

Day 6 – Day 18 of gestation; Vehicle: medium chain triglyceride.

Findings: Effects on Dams

There were no deaths or abortions in any group. All groups, including the control group, demonstrated decreased feces on Day 24-28 of gestation (1-3 animals/group). There was no dose-response relationship to this finding and was determined to be incidental. No significant differences between control and treated groups in group mean body weight on any day or body weight gain during or after test article administration occurred. Following the treatment period, 1 or 2 animals in each group, including the control group, had a decrease in body weight. This finding was determined not to be dose-related and to be incidental.

No statistical differences in group mean food consumption were observed except for a significant low value in the low-dose group on Day 1 (prior to test article administration).

Discoloration of the liver was observed in one animal each in the low- and high-dose groups. This finding was determined not to be dose-related and to be incidental.

No significant differences in the number of *corpora lutea*, number of implantations or index of implantations between the control group and treated groups were observed.

Findings: Effects on Embryo/Fetal Development

No significant differences in the number or index of embryo-fetal deaths, the number of live fetuses (male/female), sex ratio, or body weight of live fetuses (male/female) were observed between the control group and treated groups. There were no gross pathological abnormalities of the placenta observed in any group.

Complicated abnormality (hypoplasia of the face and body, thoracogastroschisis, meningoencephalocele, and spina bifida) and ectrodactyly in the right forelimb were each observed in 1 fetus in the control group. Bilateral paw hyperflexion in forelimb or hindlimb were each observed in mid- or high-dose groups. No significant differences in the index of external abnormalities between the control group and treated groups were observed. These findings were determined to have no dose-relationship and to be incidental.

Visceral examination revealed no significant differences between the control group and treated groups in the index of visceral abnormalities or variations with the exception of a significant low value in the low-dose group. These findings were determined to have no dose-relationship and to be incidental.

There were no significant differences in the index of skeletal abnormalities, variations, or the progress of skeletal ossification between the control group and any treated group.

Conclusion

The study concluded the NOAEL for general toxicity on dams, reproductive functions of dams and embryo-fetal development was 1000 mg/kg/day. (Bernard et al. 2008b)

Study Evaluation:
Standard study design employed; no toxicity was observed.

C. Toxicology Studies Performed Using "Laboratory Scale" Samples

1. Genotoxicty

Bacterial Reverse Mutation Test With and Without Metabolic Activation
S. typhimurium and *E. Coli*; TA100, TA1535, TA98, TA1537, WP2*urvA*; Dose: 0 - 1000 µg/plate without metabolic activation.

Results: Positive

Administration of the test article in strain TA100 produced a dose-dependent and two-fold or more increase in the number of revertant colonies as compared to the negative control group. The number of revertant colonies in TA98 increased by 1.77-fold when compared to negative control group. No increase in revertant colonies was observed in the other strains tested without metabolic activation. (Bernard et al. 2008a)

S. typhimurium and *E. Coli*; TA100, TA1535, TA98, TA1537, WP2*urvA*; Dose: 0 - 2500 µg/plate with metabolic activation (S9)
Results: Negative

No increase in the number of revertant colonies was observed in any strain under these test conditions. A growth inhibitory effect was observed in all strains at 2500 µg/plate or more. The positive control produced markedly induced reversion in each test strain. (Bernard et al. 2008a)

Study Evaluation:

Standard study design employed; used concurrent positive and negative controls, demonstrated toxicity at top dose.

Chromosome Aberration Test (Chinese Hamster Lung Fibroblast Cell Lines (CHL/IU)

CHL/IU; Dose: 0-194 µg/mL, without metabolic activation; 6 hr exposure; Results: Equivocal. (Bernard et al. 2008a)

CHL/IU; Dose: 0-1500 µg/mL, with metabolic activation (S9); 6 hr exposure; Results: Negative. (Bernard et al. 2008a)

CHL/IU; Dose: 0-194 µg/mL, without metabolic activation; 24 hr exposure; Results: Positive. (Bernard et al. 2008a)

Study Evaluation:

Standard study design employed; tested with and without metabolic activation, obtained positive results.

Mouse Micronucleus Test

Mouse, male BDF_1; 5/group, gavage; Dose: 0, 500, 1000, 2000 mg/kg, 2 days; Vehicle: medium chain triglyceride; Results: Negative. (Bernard et al. 2008a)

Study Evaluation:

Standard study design employed; Highest recommended dose was employed (2000 mg/kg)

Single Cell Gel Electrophoresis (SCG) Assay

Rat, male Sprague-Dawley; 4/group, gavage; Dose: Negative control, positive control (ethyl methanesulphonate [EMS]), 1000, 2000 mg/kg, 2 days; Vehicle: medium chain triglyceride; Target tissues: liver, kidney, intestinal tract (duodenum).

Findings: Body Weight and Clinical Signs

There were no significant differences between the negative control and either treatment group. The high-dose group demonstrated a slight and non-significant decrease in body weight prior to tissue sampling. No other toxicological signs were observed.

Findings: SCG assay; Liver

In the negative control group, mean Olive tail moment was 0.245 and the mean % tail DNA was 2.467.

In treated groups, three hours after the second dose, mean Olive tail moments were 0.283 for the low-dose group and 0.422 for the high-dose groups (1.16 and 1.72 times the negative control values, respectively). The mean % tail DNA in the treated groups was 2.750 (low-dose group) and 3.864 (high-dose group). Both parameters in the high-dose group were significant increases ($0<0.05$) as compared to the negative control group.

The EMS-treated group (positive control), at 3 hours post-dosing exhibited tail moment and mean % tail DNA of 1.390 and 10.578 (5.67 and 4.29 times the negative control values, respectively). Statistically-significant increases were observed.

Findings: SCG assay; Kidney

In the negative control group, mean Olive tail moment was 0.451 and the mean % tail DNA was 4.824.

In treated groups, three hours after the second dose, mean Olive tail moments were 0.610 for the low-dose group and 0.596 for the high-dose groups (1.35 and 1.32 times the negative control values, respectively). The mean % tail DNA in the treated groups was 5.816 in the low-dose group and 5.680 in the high-dose group (1.12 and 1.18 time the negative control values, respectively). While Olive tail moment was significantly increased in both treatment groups and the mean % tail DNA in the low-dose group demonstrated significant increases as compared to control, these increases were considered not to be of biological significance due the distribution of the data and a lack of a dose relationship.

The EMS-treated group (positive control) at 3 hours post-dosing exhibited mean Olive tail moment and mean % tail DNA of 2.036 and 12.761 (4.51 and 2.65 times the negative control values, respectively). Statistically-significant increases were observed.

Findings: SCG assay; Intestinal Tract

In the negative control group, mean Olive tail moment was 0.309 and the mean % tail DNA was 3.950.

In treated groups, three hours after the second dose, mean Olive tail moments were 0.328 for the low-dose group and 0.533 for the high-dose groups (1.06 and 1.72 times the negative control values, respectively). The mean % tail DNA in the treated groups was 4.096 in the low-dose group and 5.745 in the high-dose group (1.04 and 1.45 times that of the negative controls). Both parameters in the high-dose group were significantly increased ($0<0.05$) as compared to the negative control group.

The EMS-treated group (positive control), at 3 hours post-dosing exhibited mean Olive tail moment and mean % tail DNA of 1.401 and 13.417 (4.53 and 3.40 times the negative control values, respectively). Statistically-significant increases were observed.

Conclusion:
Slight increase in DNA damage was observed in liver and intestinal tract of the high-dose group as compared with the negative control group. Slight increase in DNA damage in the kidney was observed only in the low-dose group and was determined not to be dose related. The study concluded the results were equivocal due to small increases in values. (Bernard et al. 2008a)

Study Evaluation:
Standard study design, concurrent positive and negative controls employed; highest dose referred to the OECD in vivo genotoxicity guideline employed (2000 mg/kg).

2. Subchronic (13-Week Study) Rat Toxicty

Rat, Sprague-Dawley; 10/sex/group, gavage, 13 weeks, Doses: 0, 100, 300, 1000 mg/kg, Vehicle: medium chain triglyceride.

Findings:
There were no test article related changes in clinical signs, body weight, food/water consumption, urinalysis, ophthalmology or hematological values and histopathology as compared to controls.

Swelling of the fore or hind limb was observed in one low-dose male and one mid-dose female. This finding was determined to be unrelated to treatment.

There were a number of incidental findings that were determined to be unrelated to test article administration. These included:

a) In mid-dose females - a significant increase in water intake compared to controls on day 4, and an increase in urea nitrogen.

b) In mid-dose males – an increase in the percentage of lymphocytes and a decrease in the percentage of segmented neutrophils.

c) In high-dose males – an increase ALT activity

d) In high-dose females – an increase in calcium, percentage of albumin fraction and A/G ratio and a decrease in the percentage of γ–globulin fraction.

e) In high-dose males and females – an increase in total protein, and an increase in liver weight without corresponding changes in the microscopic observations.

Conclusions:

Histopathological examination did not reveal any abnormalities or test article-related changes in the liver. The increase in ALT was determined to be within the range of the control values. Therefore the study concluded the statistically-significant changes in liver weight and increased ALT (IU/L) were not related to test article administration. The study determined the NOAEL was 1000 mg/kg/day. (Kodama et al. 2008c)

Study Evaluation:

Standard study design employed; and no toxicity was observed.

3. Pharmacokinetic Studies

Pharmacokinetic Study of ^{14}C-dihydrocapsiate in Rats

The pharmacokinetics after a single oral administration (po) of ^{14}C-dihydrocapsiate at a dose of 10mg/11.6MBq/kg body weight was evaluated in fasting male Sprague-Dawley rats.

The plasma from 3 rats (single sample/time point) was examined at 5, 15, 30 minutes, 1, 2, 4, 6 and 8 hr after dosing. The radioactivity concentration in the plasma reached a maximum of 1870 ng eq./mL at 0.67 hr and subsequently declined with an apparent $t_{1/2}$ of 2.4 hr. The $AUC_{(0-\infty)}$ was 7581 ng eq·hr/mL.

The urine, feces, expired air and carcass (single sample/time interval or time point) were collected and examined (in 3 rats) at the following intervals or time points: urine and expired air (0 – 4, 4 – 8, 8 – 24, 24 – 48 and 48 – 72 hr after dosing), feces (0 – 24, 24 – 48 and 48 – 72 hr after dosing), carcass (72 hr after dosing). The excretion of radioactivity in the urine, feces and expired air were 78.2%, 19.4% and 0.5% of the dose up to 72 hr after dosing, respectively. The residual radioactivity in the carcass was 4.0% of the dose at 72 hr after dosing.

The bile, urine, feces, gastrointestinal contents and carcass (single sample/time interval or time point) were collected and examined (in 3 rats) at the following intervals or time points: bile (0 – 2, 2 – 4, 4 – 8, 8 – 24 and 24 – 48 hr after dosing), urine and feces (0 – 24 and 24 – 48 hr after dosing), GI contents (48 hr after dosing), carcass (excluding GI contents, 48 hr after dosing). The excretion of radioactivity in the bile, urine and feces were 3.4%, 68.2% and 6.0% of the dose up to 48 hr after dosing, respectively. The residual radioactivity in the GI contents and carcass were 5.6% and 14.3%, respectively.

Distribution in tissues (25 examined) was obtained from three rats at each of 5 time points (15 and 30 minutes, 2, 6 and 24 hr) after oral administration. The radioactivity concentrations in the tissues reached maxima at 30 minutes in the fat and small intestines, at 6 hr in the stomach and large intestine and at 2 hr in the other tissues. The radioactivity concentrations which were higher than that in the plasma were observed in the kidney, liver, stomach, small intestine and large intestine.

At 6 hr, plasma levels had decreased to 15% of the maximum value at 2 hr, and all tissue levels (except digestive tract) had decreased. The concentration of radioactivity in the kidney was the highest, being 6.49 times that in the plasma.

In the plasma at 15 minutes after dosing, dihydrocapsiate was not detected, while main metabolites RP2, RP3 and RP4 accounted for 23.8%, 46.4% and 2.8% of the radioactivity in the plasma, respectively.

In the plasma at 30 minutes and 2 hr after dosing, dihydrocapsiate was not detected while RP2 and RP3 accounted for $\geq$15.5% and $\geq$49.4% of the radioactivity in the plasma, respectively. In the plasma at 6 hr after dosing, dihydrocapsiate was not detected in the plasma, while RP2 and RP3 accounted for 21.4% and 20.8% of the radioactivity, respectively.

On the HPLC radiochromatograms of the 30 minutes and 2hr plasma after treatment with β-glucuronidase/arylsulfatase, RP2, RP3 and RP4 disappeared, whereas vanillyl alcohol and vanillic acid were detected. Hydrolysis of only RP2 was inhibited by addition of β-glucuronidase inhibitor, suggesting that RP2 is a glucuronide of vanillyl alcohol, RP3 is a sulfate of vanillyl alcohol, and RP4 is a sulphate of vanillic acid. (Bernard et al. 2009)

Inhibitory activity of capsinoids on CYP3A4

The inhibitory activity of capsaicin, capsiate, dihydrocapsiate and nordihydrocapsiate on CYP3A4 were examined. Capsaicin demonstrated a significant inhibitory effect on CYP3A4, having an IC_{50} value of 21.5 µmol/L and likely to be a mechanism based inhibition. The other compounds with a concentration of 100µmol/L showed no inhibitory activity. (Takanohashi et al. 2008)

D. Studies Performed Using CH-19 Sweet Extract

CH-19 Sweet Extract is the extracted oil from CH-19 Sweet (AMA), a non-genetically modified sweet chili pepper cultivar derived from self-progeny of CH-19 (parent cultivar), commonly eaten in Thailand. The refined and concentrated oil is a mixture of capsinoids including capsiate, dihydrocapsiate and nordihydrocapsiate. The concentration of capsinoids in CH-19 Extract is approximately 7.5% (7.0% - 8.0%). The primary capsinoids, capsiate, dihydrocapsiate, and nordihydrocapsiate are present in proportions of approximately 7:2:1.

A New Dietary Ingredient Notification of CH-19 Sweet Extract was submitted to FDA by the applicant, and the FDA accepted the dietary ingredient notification for their file on May 17, 2007.

As additional supporting information, below are summaries of studies conducted employing the naturally occurring CH-19 Sweet extract. The level of dihydrocapsiate, the compound under consideration, is identified for each study.

1. Acute Toxicity

Rat, Sprague-Dawley, 5/sex/group, gavage, single dose, Doses: 0, 5, 10, 20 mL/kg (approximately 71.25, 142.50, 285 mg/kg dihydrocapsiate), Vehicle: medium chain triglyceride, LD50=>20 mL/kg (approximately 285 mg/kg dihydrocapsiate). Soft stools were interpreted to be from the large amount of the oily vehicle. (Watanabe et al. 2008b)

Study Evaluation:
Standard limit test design, dose and observation period, limited by the low concentration of dihydrocapsiate

2. Subchronic (13-Week Study) Rat Toxicity

Rat, Sprague-Dawley, 10/sex/group, gavage, 13 weeks, Doses: 0, 1.25, 2.5, 5.0 mL/kg/day (equivalent of 16.63 to 20.19, 33.25 to 40.38,and 66.5 to 80.75 mg/kg/day dihydrocapsiate).

Findings:
Statistically-significant decreased MCV and MCH observed in top-dose females, interpreted as having no toxicological significance as changes were minimal (lower than control by $\leq$4%) and due to the lack of changes red blood cell count, hematocrit, or hemoglobin and lack of abnormalities in hematopoietic organs upon histopathological examination .

Mid- and high-dose females demonstrated significant prolongation in prothrombin time (mid-dose 13.9$\pm$0.4 sec, range 12.9 – 14.5 sec; high-dose 14.0$\pm$0.5 sec, range 13.2 - 14.6 sec). These findings were interpreted as not being treatment related as they were only slightly higher that the range for the control group (12.6 – 14.1 sec). No changes in any other coagulation parameters were observed

and no tendency to hemorrhaging was observed during the histopathological examination.

One male in the high-dose group demonstrated increases in AST and LDH values and statistically-significant increases in ALT activity. Focal necrosis and bile duct proliferation in the liver were found on histopathological examination. Increased liver weights were observed in the high-dose group. Therefore the increase in the one male was considered treatment related.

A Pathology Working Group reviewed the histopathological findings of increased incidence and degree of focal myocarditis in males in all groups, including the control group. The PWG concluded there is no evidence the myocarditis was chemically-induced and the incidence and severity of lesions demonstrated were comparable to those expected from spontaneous cardiomyopathy. (See Section 5.6.3.5 for a discussion of the PWG process and findings)

Conclusion:
Based on these findings, the NOAEL in males is 2.5 mL/kg/day and 5.0 mL/kg/day in females (33.25 to 40.38 mg/kg/day in males and 66.5 to 80.75 mg/kg/day dihydrocapsiate in females) (Kodama et al. 2008a)

Study Evaluation:
Standard study design employed; toxicity was observed. Better than usual having been reviewed by a PWG.

3. Chronic (26-Week Study) Rat Toxicity

Rat, Sprague-Dawley, 20/sex/group, gavage, 26 weeks, Doses: 0, 1.25, 2.5, 5.0 mL/kg/day (equivalent of 16.63 to 20.19, 33.25 to 40.38, and 66.5 to 80.75 mg/kg/day dihydrocapsiate).

Findings:
Females in the high-dose group demonstrated a significant increase of chloride in urine and a decrease of chloride in blood chemistry; the elevated level of chloride was determined not to be treatment related due to the lack of other abnormal renal function parameters and the lack of abnormalities upon histopathological examination.

Significantly high values were observed in the percentage of segmented neutrophils in high-dose males and significantly low values in the percentage of eosinophils in high-dose females. The low value of eosinophils was determined not to be treatment related as the individual percentage and individual absolute number were within the range of the control group. The low value in the percentage of lymphocytes was observed in high-dose males; however it was not different in the absolute number as compared to controls.

All treated males demonstrated significant decreases in PT and APTT. These findings were determined not to be toxic since the results reflect shortening of PT and APTT and not prolongations. There was no associated pathological change and the decrease in APTT was not dose-related.

Although significant increases in the excretion of sodium was observed in mid- and high-dose males and high-dose females and potassium in high-dose females, there were no changes in associated blood chemistry values. Thus findings were determined not to be treatment related.

Significantly high or high values were observed in AST, ALT and LDH in high-dose males.

Focal liver necrosis was observed in one male in the control, low- and mid-dose groups and in four males in the high-dose group. Myocardial fibrosis was diagnosed in 14 males and 5 females in the control group, 13 males and 8 females in the high-dose group. Focal myocarditis was observed in 18 males and 15 females in the control group, and 17 males and 14 females in the high-dose group.

A Pathology Working Group reviewed these histopathological findings and concluded there is no evidence the myocarditis was chemically-induced and the incidence and severity of lesions demonstrated were comparable to those expected from spontaneous cardiomyopathy. (See Section 5.5.3.5 for a discussion of the PWG process and findings)

Conclusion:
Based on these findings, the NOAEL in males is 2.5 mL/kg/day and 5.0 mL/kg/day in females (33.25 to 40.38 mg/kg/day in males and 66.5 to 80.75 mg/kg/day dihydrocapsiate in females). (Kodama et al. 2008a)

Study Evaluation:
Standard study design employed; toxicity was observed; better than usual having been reviewed by a PWG.

4. Two-Generation Reproduction Study in the Rat

Rat, Sprague-Dawley, two generations, 24/sex/group in the F_0 generation, gavage, Doses: 0, 1.25, 2.5, 5.0 mL/kg/day (equivalent of 14.25 to 20.19, 28.5 to 40.38, and 57 to 80.75 mg/kg/day dihydrocapsiate). Vehicle: medium chain triglycerides.

Findings: F_0 Generation

There were no test article-related deaths and no toxicological abnormalities in body weight, food consumption, clinical signs or gross pathological findings. There were no treatment related effects on the number of estruses, estrous cycles, copulation index, the number of days before copulation, fertility index, number of implantations, gestation period, number of live pups, delivery index, still birth or live birth index, or in nursing conditions.

Transient salivation following dosing was observed in all groups, including the control group. This effect was considered to be of no toxicological significance.

No statistically-significant differences in body weights of males and females as compared to controls were observed except in mid-dose females during the pre-mating period. In the low-dose group, increased food consumption was observed in males from Day 36 onward, as compared to controls.

One male in the high-dose group died from aspiration of ingesta and classified as an accidental death. The stillbirth index was significantly lower in the low-dose group and the number of implantations and stillbirth index were significantly lower in the mid-dose group as compared to controls. There were no significant differences in the number of implantations and still birth index in the high-dose group as compared to controls.

One male in the mid-dose group was observed to have a focal adhesion of the lung to the thoracic wall and opacity of the pericardium. In the control group, one male presented with bilateral smallness of the testes and epididiymis.

Findings: F_1 Generation

There were no test article related effects on sex ratio at birth or body weights of live pups, and no external abnormalities.

The low- and high-dose groups demonstrated significantly lower index of eyelid opening on Day 14 after birth. This was determined not to be treatment-related since eyelid opening was observed in all animals by Day 17 and there was nothing suggestive of growth retardation (i.e., changes in body weight). The incisor eruption index on Day 11 after birth was significantly higher in the low- and mid-dose groups. No significant differences were observed in the index of pinna detachment, opening of vagina or cleavage of the balanopreputial gland between controls and any test group.

Necropsy of pups that died revealed no test article-related gross abnormalities of the main organs in the thoracic and abdominal cavities. Two males and one female in the control group, one male and three females in the mid-dose group and one female in the high-dose group presented with thymic remnant in the neck on Day 4 after birth. There were no other organ abnormalities in the thoracic or abdominal cavity; this finding was determined to be incidental. One male in the low-dose group at weaning was found to have thymic remnant in the neck. Necropsy of stillborn pups revealed bilateral ureteral dilatation in one female in the high-dose group. No other macroscopic abnormalities were observed in the main organs or tissues of the thoracic or abdominal cavities; this finding was determined to be unrelated to treatment.

Preyer's reflex was normal in all groups. Mid-dose males and females demonstrated significantly higher air righting reflex index as compared to controls. Reaction time of righting reflex was not significantly different in any of the groups.

The control, the low- and mid-dose groups demonstrated prone position beginning at one and two weeks of treatment. Similar to F_0 generation, sporadic increased salivation was observed in males and females in the mid- and high-dose groups.

High-dose males had significantly higher body weight as compared to controls from Day 56 after birth and on. As compared to controls, low-dose males showed significantly higher body weight from Day 133 after birth onward. The mid-dose male body weight was not significantly different from controls. No significant differences were demonstrated in female body weight or body weight gain from the day of birth to the starting day of mating, during gestation period or during lactation in any of the groups.

No significant differences were demonstrated in the number of estruses, estrous cycle, copulation index, the number of days until copulation, fertility index, number of implantations, gestation period, number of live pups, delivery index, stillbirth or live birth index in any of the groups.

One dam in the mid-dose group has insufficient lactation on Day 2 that resulted in a complete loss of the pups. This finding was observed in only this one animal and no similar abnormalities were found in any other treatment groups.

One male in each of the control, mid- and high-dose group showed a dark red area of the liver. An enlarged thymus was observed in one mid-dose male.

One female in the low-dose group that died presented a dark area in the lung and an enlarged spleen, liver, and adrenal glands. No macroscopic abnormalities in other organs in the thoracic or abdominal cavity in any other tested animal.

Focal myocarditis was found in 15, 17, 18, 17 males and myocardial fibrosis in 2, 4, 2, and 5 males in the control, low-, mid- and high dose, respectively. These findings were addressed by the Pathology Working Group; the PWG concluded that there was no evidence that these findings were test article related. (See Section 5.5.2.6 for a discussion of the PWG process and findings)

Findings: F_2 Generation

There were no significant differences in sex ratio at birth or body weight and no external abnormalities.

The top-dose group demonstrated a significantly higher index of eyelid opening on Day 14 after birth, as well as air righting reflex as compared to controls. As with the previous generation, there was no suggestion of growth disturbances (i.e., body weight). No significant differences were observed in any of the groups with regard to pinna detachment or incisor eruption, the reaction time of righting reflex or index of Preyer's reflex. No macroscopic abnormalities of the main organs and tissues of the thoracic or abdominal cavities were observed. On Day 4 after birth, gross pathology examination reveled one female in the low-dose group demonstrated opacity of the right medial lobe of the liver, and thymic remnant in the neck in two females in the high-dose group. No other abnormalities were observed. In gross pathology at weaning, microphthalmia was observed in one male and one female in the low-dose group. One female in this group demonstrated cecal obstruction. No other macroscopic abnormalities were observed.

Conclusion:

The report concluded that the NOAEL for CH-19 Sweet Extract was 5.0 mL/kg/day (the equivalent of 57 to 80.75 mg/kg/day dihydrocapsiate). (Kodama et al. 2008b)

Study Evaluation:

Standard study design employed; in the absence of toxicity at the high dose, had this been a *drug,* a higher dose would have been suggested; this is problematic given the low concentration of dihydrocapsiate in the extract.

5. Teratology in Rats

Rat, Sprague-Dawley, 20 pregnant dams/group, gavage, Dosing Day 7 to Day 17 of gestation, Doses: 0, 1.25, 2.5, 5.0 mL/kg/day (equivalent of 20.19, 40.38 and 80.75 mg/kg/day dihydrocapsiate) Vehicle: medium chain triglycerides.

Findings:

There were no deaths, nor dams with premature delivery/abortion in any groups. The only abnormality observed during gestation was salivation in two females in the high-dose group on Day 17 of gestation. The mid-dose group demonstrated a significant increase in body weight gain on Day 18 to Day 20 of gestation. The low- and mid-dose groups had significant increases in food consumption from Day 14 to Day 18 of gestation as compared to controls. Food consumption tended to be higher in the high-dose group as compared to controls during this period. These findings were only observed during Days 14-18 of gestation. Gross pathology examination revealed no findings in any group. No significant differences were observed in the number of corpora lutea, number of implantations, implantation index, index of dead embryos/fetuses, or number of live fetuses.

No significant differences in sex ratio or body weight of live fetuses were observed in any group. For external abnormality, short trunk with vestigial tail was observed in one fetus in the high-dose group. No macroscopic abnormalities in the placenta of live fetuses were observed in any group and placental weights were comparable across all groups. Visceral abnormalities and variations were observed in fetuses from all groups; however, all incidences were comparable between controls and test groups. The abnormalities observed were dilatation of lateral ventricle, abnormal origin of the left pulmonary artery, ventricular septal defect, and abnormal lobulation in the liver. The variations observed were thymic remnant in the neck dilatation of renal pelvis and dilatation and convolution of the ureter. One skeletal abnormality was observed in 1 fetus in the low-dose group. Although skeletal variations were observed in all groups, there were no significant differences in incidences between control and treated groups. Skeletal variations were observed in the cervical rib, 14^{th} rib, splitting of thoracic vertebral body, splitting of sternebrae, and lumbarization of sacral vertebra. There were no significant differences in the number of ossified metacarpi, ossified metatarsi, ossified sacral and caudal vertebrae, or in the index of ossified sternebrae between the control and any test groups.

Conclusion:

The study concluded the NOAEL for this study is 5.0 mL/kg/day (80.75 mg/kg/day dihydrocapsiate). (Bernard et al. 2008c)

Study Evaluation:

Standard study design employed; in the absence of toxicity at the high dose, had this been a *drug,* a higher dose would have been suggested; this is problematic given the low concentration of dihydrocapsiate in the extract.

6. Embryo Fetal Development in Rabbits

Rabbit, female New Zealand white, 17 to 22 pregnant dams/group, gavage, Dosing Day 6 to Day 18 of gestation, Doses: 0, 0.25, 0.5, and 1 mL/kg/day (3.8, 7.6, 15.2 mg/kg/day dihydrocapsiate), Vehicle: medium chain triglycerides.

Findings:

There were no deaths in any of the test groups; one female in the low-dose group aborted the fetus. Decrease in feces, similar to that of the control group, was exhibited in all test groups. There were no significant differences in group mean body weight or group mean body weight gain. Individual animals demonstrated decreased body weight gain (1 in control group, 2 in low-dose group, and 3 in the high-dose group), however there was no dose-related tendency toward an increase magnitude of the decrease in body weight. No statistically-significant differences in group mean food consumption were noted. Individual animals showed a low value of a one-day food consumption (20 g or less). There were no dose-related increases in the incidence or duration.

Gross pathology revealed no abnormalities in any of the four groups or in the aborted fetus. There were no significant differences in treated groups as compared to controls in the number of corpora lutea, number or index of implantations, or number of index of embryo-fetal deaths.

No significant differences were observed in the number of male or female live fetuses, sex ratio, or body weight of male or female fetuses. No gross pathological abnormalities in the placenta or no changes in placental weight were observed.

For external abnormality, meningocele and syndactyly in the forelimb, each in one fetus in the control group, cleft of abdominal wall in one fetus of the mid-dose group and club foot in one fetus of the high-dose group were observed. These external abnormalities did not represent a significant difference in incidence of treated groups as compared to controls.

Visceral abnormalities and variations and skeletal abnormalities and variations were observed in all groups. However, there was no significant difference in the number of live fetuses with these changes between controls and treated groups. The incidence of thymic remnant in neck, a visceral variation, and the incidence of unossified talus, a skeletal variation, were significantly higher in the top-dose group than controls (thymic remnant in neck: 24%; unossified talus: 3.5%). There were no other changes that suggested growth retardation, therefore these increases were determined to be incidental and not related to test article administration.

Conclusion:
The study concluded the NOAEL for this study is 1.0 mL/kg/day (15.2 mg/kg/day dihydrocapsiate). (Bernard et al. 2008c)

Study Evaluation:
Standard study design employed; a slightly higher number of litters would have been preferable, in the absence of toxicity at the high dose, had this been a *drug,* a higher dose would have been suggested; this is problematic given the low concentration of dihydrocapsiate in the extract.

7. Review by Pathology Working Group

CH-19 Sweet Extract contains capsinoids as functional components. The capsinoids are derived from extracted oil of Sweet Chili Peppers (*Capsicum annuum* L.) and are intended to be marketed as a dietary supplement in the form of soft gel capsules. On October 2, 2006 the FDA returned comments on a New Dietary Ingredient Notification submitted by the applicant. The FDA indicated that it had significant concerns about the evidence presented to support the conclusion that a dietary supplement containing "Capsinoids will reasonably be expected to be safe." The FDA communication specifically commented on results from the 13-week oral toxicity study of CH-19 Sweet Extract (Study Number N-B180). The FDA stated that "the information provided from this study raises concerns about cardiac toxicity of 'Capsinoids (Extracted Oil of Sweet Chili Peppers)' and was inadequate to allow FDA to evaluate the basis for the safety of your product. For example, there were highly significant, treatment-related increases in the incidence and degree of focal myocarditis in male rats in each test substance group."

Based on the situation above, a Pathology Working Group (PWG) was established and reviewed heart sections independently from the applicant. The findings of this PWG were submitted to FDA by the applicant, and after reviewing its findings, the FDA withdrew its objections and accepted the dietary ingredient notification for their file on May 17, 2007.

Members of the PWG

The Pathology Working Group was chaired by Dr. Henry Wall, Experimental Pathology Laboratories, Inc. (EPL®), who organized and presented the material to the panel of five pathologists with specific expertise in the cardiovascular system and/or with regulatory toxicologic pathology. The PWG Chairperson and all experts selected as PWG Participants were veterinary pathologists certified by the American College of Veterinary Pathologists. Individuals participating in the PWG or attending as an observer are listed as follows:

Dr. Henry G. Wall	Chairperson
Dr. W. Ray Brown	PWG Participant
Dr. Charles B. Clifford	PWG Participant
Dr. Jerry F. Hardisty	PWG Participant
Dr. Ernest E. McConnell	PWG Participant
Dr. Paul W. Snyder	PWG Participant
Dr. George Burdock	Observer
Dr. Yoshiyuki Fujishima	Observer

PWG Method and Discussion

The PWG was provided with all the heart section microscopic slides from the 3 studies (both male and female). The PWG examined coded slides without knowledge of treatment group and previous diagnosis. The animals were randomized using a computerized random number generator before they were coded. After an initial round of review of hearts from 20 rats with changes representing the spectrum of diagnoses made by the Study Pathologist, the PWG discussed terminology and concluded that myocarditis and myocardial fibrosis were part of the continuum of changes that is commonly referred to as cardiomyopathy (Jokinen et al. 2005; MacKenzie and Alison 1990).

Studies Reviewed

A Pathology Working Group (PWG) Review was performed to review the heart sections from two oral toxicity studies and a reproduction study conducted in rats with CH-19 Sweet Extract. These studies are listed as follows:

- A 13-Week Oral Toxicity Study of CH-19 Sweet Extract in Rats (Study Number N-B180)
- A 26-Week Oral Toxicity Study of CH-19 Sweet Extract in Rats (Study Number N-B145)
- A Reproduction Study of CH-19 Sweet Extract By Oral Administration in Rats (Study Number N-R008)

PWG Charge

The purpose of this review was to have a panel of pathology experts examine heart sections to evaluate the findings reported by the Study Pathologist and to provide a consensus diagnosis for the heart for each animal evaluated. In addition, the PWG provided its consensus diagnoses for heart sections of groups of male rats in the 13-week study and male and female rats in the 26-week study that were not evaluated by the Study Pathologist for each study. The PWG specifically focused on the presence or absence of heart microscopic morphologic changes that might be consistent with myocarditis, myocardial fibrosis or the continuum of degenerative and inflammatory myocardial changes commonly referred to as cardiomyopathy. The PWG review was conducted using procedures similar to those followed routinely by the pharmaceutical companies and regulatory authorities. The PWG panel was also requested to provide a discussion on the toxicological significance of the microscopic changes in the heart of rats orally dosed with CH-19 Sweet Extract.

The PWG also considered that the use of a single term would also more accurately reflect incidence of lesions that represented a continuum of related morphologic effects. The PWG findings for each animal were discussed by the group, reexamined if necessary to assure that all pertinent structural features bearing on the diagnosis were considered by all PWG panel members, and the final opinions were recorded on the Chairperson's worksheets. The consensus diagnoses of the PWG were reached when at least three of the five PWG participants were in agreement.

PWG Conclusions

The PWG concluded that:

1. The myocardial lesions observed in animals that received CH-19 Sweet Extract are consistent in structural character, distribution, and severity with myocardial lesions associated with spontaneous cardiomyopathy, an age-related progressive condition that is common to the Sprague-Dawley, Wistar and Fischer 344 rat strains (Jokinen et al. 2005; Kemi et al. 2000; Lewis 1992; Ruben et al. 2000).

2. The differences in incidence and severity with dose and duration of dosing as evidenced in the three studies do not provide evidence of a pattern of widespread myocardial injury as would be expected for chemically-induced myocarditis.

3. The myocardial alterations observed in rats orally administered CH-19 Sweet Extract in these studies are not toxicologically significant since the incidence and severity of lesions are comparable to those expected for spontaneous cardiomyopathy.

8. Genotoxiciy

Bacterial Reverse Mutation Test, with and without metabolic activation (S9), TA 100, TA98, TA1535 and TA1537 and WP2*uvrA*; Dose range: 0.819 to 5000 µg/plate. Negative in the presence and absence of metabolic activation. (Watanabe et al. 2008b)

Study Evaluation:
Standard study design employed; tested with and without metabolic activation

Chromosome Aberration Test, with and without metabolic activation (S9), short term and continuous 24-hr treatment, Chinese hamster lung fibroblast cell line (CHL/IU), Dose range: 1201 to 5000 µg/mL. Negative in the presence and absence of metabolic activation. (Watanabe et al. 2008b)

Study Evaluation:
Standard study design employed; tested with and without metabolic activation

Micronucleus Test, male mouse (BDF_1), 5/group, gavage, once daily for two days, Doses: one control (no treatment), one positive control (MMC via ip administration), one negative control, 5, 10, 20 mL/kg. Results: Negative (Watanabe et al. 2008b)

Study Evaluation:
Standard study design employed

9. Human Study

Safety Assessment and Pharmacokinetics of Capsinoids in Healthy Male Volunteers after a Single Oral Ingestion of CH-19 Sweet Extract

The safety and pharmacokinetics of a single oral dose of capsinoids were evaluated in 24 healthy, fasted Japanese male volunteers (20-38 yrs), using a randomized, placebo-controlled, double-blind, parallel group study. Subjects received either 3 or 6 capsules, contained 15 or 30 mg of capsinoids, respectively (equivalent of 3.96 mg and 7.92 mg of dihydrocapsiate, respectively).

Subjects were evaluated on the following: physical examinations, adverse events, laboratory tests (hematology, blood chemistry, and urinalysis), body weight, vital signs (blood pressure and heart rate), ECG and body temperature, plasma concentrations of capsinoids and vanillyl alcohol, and plasma concentrations and urinary excretion of catecholamines and their metabolites.

Capsinoids were well-tolerated, and no clinically significant effects were observed even when 30mg of capsinoids was ingested. Body temperature tended to increase after ingestion. The plasma concentration of capsinoids and their metabolite, vanillyl alcohol, were below the limit of quantification. Subjects showed an increase in urinary excretion of 3-methoxy-4-hydroxyphenylglycol (MHPG), but the levels were not statistically different from the Placebo Group. (Bernard et al. 2008d)

Study Evaluation:

Preliminary human study, limited number of subjects, no effects observed, substances below LOQ, finding the LOEL would be of interest in subsequent studies

10. Pharmacokinetics Studies

Plasma concentration-time profile of capsinoid and vanillyl alcohol in rats after single oral administration of capsinoids

Extracted capsinoids (10 and 100 mg/kg) were orally administered (po) to fasted male Sprague-Dawley rats. The concentration profiles of capsinoids (capsiate, dihydrocapsiate and nordihydrocapsiate) and vanillyl alcohol in the portal vein plasma and systemic plasma were examined at 5, 15 and 30 minutes, 1, 2 and 4 hr (3 rats/time point after dosing).

Capsiate, dihydrocapsiate and nordihydrocapsiate were below the limit of quantification in either the portal vein plasma or the abdominal aorta plasma at either dose or at any time point.

The C_{max} for vanillyl alcohol in the portal vein plasma was 0.163 and 1.48 µg/mL at 10 and 100 mg/kg, respectively. Vanillyl alcohol portal vein plasma $AUC_{0\text{-}4hr}$ were 0.321 and 3.85 µg· hr/mL after 10 and 100 mg/kg, respectively. In the absence of appropriate time points reflecting an elimination phase, the $t_{1/2}$ was not calculated.

At 10 mg/kg, no vanillyl alcohol was ever detected in the systemic plasma. At 100 mg/kg, vanillyl alcohol was detected for 0.0246 µg/mL only at 5 minutes after dosing. The $AUC_{0\text{-}4hr}$ was 0.00103 µg· hr/mL but the $t_{1/2}$ could not be calculated.

These results suggest that extracted capsinoids orally administered with doses of 10 to 100 mg/kg, were most likely to be metabolized in the GI tract or alimentary mucosa or both before absorption. Vanillyl alcohol in the portal vein was probably subjected to metabolic conversion during its passage through the liver before entering into the systemic blood. (Shirai et al. 2008)

Study Evaluation:

Initial study demonstrated extremely rapid metabolism resulting in findings only at the short time interval.

Search of vanillyl alcohol conjugates using the plasma of rats after single oral administration of capsinoids

Extracted capsinoids were orally administered (po) to fasted Sprague-Dawley male rats at doses of 10 and 100 mg/kg and the plasma concentrations of vanillyl alcohol examined at 5, 15 and 30 minutes and 1, 2 and 4 hr after dosing.

At both doses, there was a tendency that the concentration of sulfate of vanillyl alcohol was higher than that of glucuronide of vanillyl alcohol. At doses of 10 and 100 mg/kg, $AUC_{0\text{-}4h}$ of sulfate was 2.27 and 18.2 μg·hr/mL, and that of glucuronide was 1.80 and 8.94 μg·hr/mL at 10 and 100 mg/kg, respectively. (Shirai et al. 2008)

Study Evaluation:

Preliminary pharmacokinetic study in rats.

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Appendix A

Appendix A

GRAS Certificate

SRA International, Inc.

CERTIFICATE OF THE EXPERT PANEL

GRAS STATUS OF

DIHYDROCAPSIATE

A panel of experts, qualified by their scientific training, national and international experience to evaluate the safety of food and food ingredients (the Expert Panel) was requested by SRA International Inc. (SRA) to determine the GRAS status of dihydrocapsiate for use in expanded food categories and higher use levels as a source of dihydrocapsiate in the diet. The Panel met at the offices of SRA International, Inc. (SRA), 5235 Ragged Point Road, Cambridge, MD 21613 on August 12, 2009.

BACKGROUND

To assist the Expert Panel in its deliberations, SRA conducted a comprehensive search of the literature on the safety of dihydrocapsiate and its components. The results of this survey were made available to the Expert Panel. The members of the Expert Panel, individually and collectively, critically evaluated the available information summarized in this document and unanimously arrived at the conclusions outlined herein (see CONCLUSIONS).

- Dihydrocapsiate is manufactured using a two-stage process. The building blocks (i.e. 8-methylnonanoic acid [MNA] and vanillyl alcohol [VOH]) are prepared separately; the former using isobutyl bromide and 6-bromohexanoic acid ethyl ester through a Grignard coupling reaction and the latter from vanillin using reduction and evaporation. Subsequently, MNA and VOH undergo esterification to produce dihydrocapsiate.

- The information critically evaluated by the Expert Panel and SRA was assembled by SRA as a dossier. This dossier contained information obtained from the Applicant in their submission for GRAS consideration (GRAS application), information obtained from the comprehensive literature search previously mentioned, and information from publicly available documents.

- The Expert Panel prepared this summary of the critical evaluation.

- A consideration of possible pharmacological effects of dihydrocapsiate was not within the purview of the Panel's review.

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POINTS CONSIDERED BY THE PANEL

The following is a limited summary of the Expert Panel's critical evaluation of the available information.

- Dihydrocapsiate, an analogue of capsaicin, is naturally occurring in a wide variety of edible non-pungent as well as pungent chili peppers and as such has been and is being consumed in the U.S. as well as world-wide.

- CH-19 Sweet Extract, of which dihydrocapsiate is a component (approx. 1.5%), has been accepted by the FDA as a dietary supplement (FDA to Applicant, May 17, 2007).

- Dihydrocapsiate contains an ester linkage not the amide linkage as found in capsaicin. This ester bond is the likely explanation for the rapid hydrolysis of dihydrocapsiate in the GI tract. The lability of the ester linkage is the likely explanation for the inability to detect dihydrocapsiate in the plasma of rats or humans.

- A range of animal and human studies demonstrate the safety of dihydrocapsiate at exposures that exceed anticipated human use levels.

- The oral administration of dihydrocapsiate, up to 1,000 mg/kg b.w./day to rats for 13- weeks, did not elicit any adverse effects.

- Dihydrocapsiate at doses up to 1,000 mg/kg b.w./day (the highest dose tested) did not elicit any adverse reproductive or developmental effects in rats.

- In the subchronic toxicity study of CH-19 (which contains approximately 1.5% dihydrocapsiate) in rats, the study pathologist raised a concern regarding cardiac changes. A PWG reviewed the heart slides from the 13- and 26-week studies and the reproductive study, and concluded that the changes represented the spontaneous cardiomyopathy of rats and were not treatment related. There was no evidence of myocarditis. The GRAS Panel agreed with these findings.

- Normal human volunteers ingested CH-19 containing 3.96 or 7.92 mg of dihydrocapsiate without the occurrence of adverse effects on clinical symptoms, physical examinations, hematology, blood chemistry, urinalysis, body weight, blood pressure, heart rate, or ECG. Neither dihydrocapsiate nor VOH were detected in the plasma.

- The food categories and use levels *previously declared GRAS by an Expert Panel (16 November 2007)* and subsequently addressed by FDA in a No Objection Letter (9 March 2009) were:

Food Group	Use Level (ppm)
Meal replacement and yogurt beverages – low-calorie	4.2
Tea sugar-free	4.2
Fruit juice – fresh orange juice	4.2
Vegetable juice	4.2
Fruit-aides, drinks, and powders – low-calorie	4.2
Beverage concentrates - low calorie	4.2
Beverage soft drinks – sugar-free	4.2
Non-carbonated water – low-calorie	4.2
Energy drinks	4.2
Yogurt – Fruit – Non and low Fat	4.4
Yogurt – Chocolate, Vanilla, Plain – Non and low fat	4.4
Oatmeal – instant, low-sugar	4.2
Chewing gum – sugarless	333
Mints dietetic, low-calorie	500
Breakfast and meal replacement bars	25
Gelatin/Puddings – low-calorie	8.3
Salad dressings – low-calorie	33
Sweeteners – low-calorie	250
Snack foods – popped-low-calorie – low fat and rice cakes	33
Frozen Desserts – light	
Frozen desserts - ice pops and fruit bars	11.8
Frozen desserts – dairy	8.3
Ready to eat meals	
Ready to eat meals – frozen diet	4.2
Ready to meals – soup	4.1
Nutritional meal – Ensure	4.2

- For those individuals consuming dihydrocapsiate in those food categories and use levels *previously declared GRAS by an Expert Panel (16 November 2007*, the intended conditions of use could result in a mean consumption of 1.8 mg/day and a 90th percentile consumption of 4.1 mg/day or 0.03 mg/kg/day and a 90th percentile consumption of 0.068 mg/kg/day for a 60- kg person.

- The food categories and use levels *declared GRAS by the current Expert Panel* were:

Food Group	Mg Dihydrocapsiate per Serving Size	FDA 21 CFR 101.12 Serving Size (g)[1]	Use Level (ppm)
Bars - Breakfast and meal replacement bars	**3.0**	40	75
Beverage concentrates[2] – low-calorie	**3.0**	21.6	138.9
Beverages soft drinks – sugar free	**3.0**	240	12.5
Chewing gun – sugarless	**10.0**	3	3333.3
Cookies – low fat and non-fat	3.0	30	100
Creamer	**3.0**	15 - 32	93.8 – 200
Energy drinks	**3.0**	240	12.5
Frozen Desserts – light	**3.0**		
Frozen desserts - ice pops and fruit bars	**3.0**	85	35.3
Frozen desserts – dairy	**3.0**	120 - 240	12.5 – 33.3
Fruit-aides, drinks, and powders – low calorie	**3.0**	240	12.5
Fruit juice – fresh orange juice	**3.0**	240	12.5
Gelatin/Puddings – low-calorie	**3.0**	120 - 147	20.4 – 25
Hard candy, dietetic (previously limited to mints)	3.0	2 - 40	75 -1500
Liquid coffee	3.0	240	12.5
Low-fat and non-fat crackers	3.0	30	100
Meal replacement beverages	**3.0**	30[3] - 240	12.5 - 100
Non-carbonated water – low calorie	**3.0**	240	12.5
Nutritional meal - Ensure	**3.0**	240	12.5
Oatmeal – instant, low sugar	**3.0**	240	12.5
Protein based meat alternative	3.0	7 - 240[3]	12.5 - 428.6
Ready to eat meals	**3.0**		

Ready to eat meals – frozen diet (non meat, non-chicken, non fish/seafood)	**3.0**	240	12.5
Ready to meals – soup	**3.0**	245	12.2
Ready to cereals, cold	3.0	15 - 55	54.5 - 200
Salad dressings – low-calorie	**3.0**	15 - 30	100 - 200
Snack foods – popped-low-calorie – low fat and rice cakes	**3.0**	30	100
Sweeteners – low-calorie	**3.0**	4	750
Tea, liquid low sugar or sugar fee	**3.0**	240	12.5
Vegetable juice	**3.0**	240	12.5
Yogurt – Fruit – Non and low Fat	**3.0**	225	13.3
Yogurt – Chocolate, Vanilla, Plain – Non and low fat	**3.0**	225	13.3

1 Title 21--Food and Drugs Chapter I--Food and Drug Administration Department of Health and Human Services.
Part 101 Food Labeling. *Reference Amounts Customarily Consumed Per Eating Occasion in the General Food Supply.*(21CFR101.12).
2 As powder, prior to being reconstituted.
3 The large range in serving sizes is due to the inclusion of foods such as imitation bacon bits and bacon strips as well as vegetarian burgers, hotdogs, etc.
4 Additions to food categories and increased use levels (since 16 November 2007) are shown in **bold**.

- For those individuals consuming dihydrocapsiate in those food categories and use levels *declared GRAS by an current Expert Panel (12 August 2009)*, the intended conditions of use could result in a mean consumption of 10.5 mg/day and a 90th percentile consumption of 22.4 mg/day or 0.175 mg/kg/day and 0.37 mg/kg/day, respectively for a 60- kg person. When calculated on the basis of U.S. per capita consumption, the estimated mean and 90th percentile are 9.3 mg/day and 21.3 mg/day, respectively.

CONCLUSIONS:

The Expert Panel unanimously concludes, based on its critical evaluation of available information, that dihydrocapsiate is safe for its intended conditions of use as previously described.

The Expert Panel further concludes that dihydrocapsiate, meeting the specifications described in the GRAS application, and used in accordance with current Good Manufacturing Practice is GRAS by scientific procedures.

(b) (6)

8/12/09

Samuel M. Cohen, M.D. Ph.D. Date

Alex Makriyannis, Ph.D. Date

Philip S. Portoghese, Ph.D. Date

William J. Waddell, M.D. Date

Bernard M. Wagner, M.D. Date

Bruce K. Bernard, Ph.D. Date
Scientific Coordinator

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Alex Makriyannis, Ph.D. Date

Philip S. Portoghese, Ph.D. Date

William J. Waddell, M.D. Date

[redacted] D. 8/12/09
[redacted] Wagner, M.D.] Date

Bruce K. Bernard, Ph.D. Date
Scientific Coordinator

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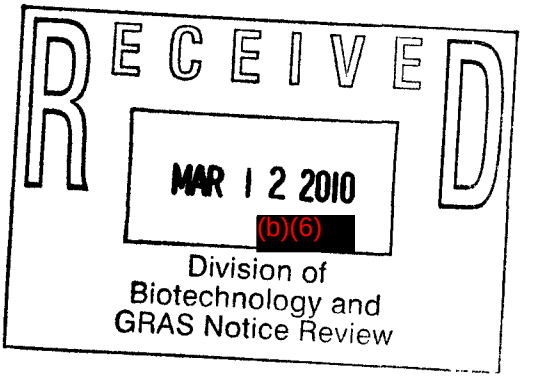
Bruce K. Bernard, Ph.D. Date
Scientific Coordinator

SUBMISSION END

AM

SRA International, Inc.

12 March 2010



Ms. Molly Harry, M.S.
Division of Biotechnology and GRAS Notice Review
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration

Re: GRN 000312 Dihydrocapsiate

Dear Ms. Harry:

This email is in response to the four (4) clarifications raised regarding GRN 000312 (Harry to Bernard, email dated 4 March 2010).

Clarification #1:

The food category listed as "protein based meat alternative" (page 3), please clarify the source of the protein.

Response to #1:
'Protein-based meat alternative' is a standard USDA food category. The source is 'vegetable and/or dairy protein'.

Clarification #2:

The reference, Kodama et. al. (2009) cited on page 37, and Bernard et. al. (2009) cited on page 50 are not listed under Section E. References. Please send us an updated list of references.

Response to #2:
The two references you cited from the text (Kodama et. al. (2009) cited on page 37; Bernard et. al. (2009) cited on page 50) were miss-cited in the text; they both should have been listed as 2008 not 2009 (please see Clarification #3 for rationale).

Clarification #3:

The following references are cited in the notice as: Shirai et al. (2008) on pages 70/71, and Takanohashi et al. (2008) on page 51. However, the list of references indicates that they were accepted for publication on December 9, 2009 in the Int J Toxicol 29(suppl), 2010. Please correct this inconsistency, to accurately reflect the current status of these publications both in the text and in the list of references.

5235 Ragged Point Road, Cambridge, MD 21613-3518
Telephone: 410. 228.1400 Telefax: 410. 228.1450

Response to #3:
The two references you cited from the text (Shirai et al. (2008) on pages 70 and 71, and Takanohashi et al. (2008) on page 51) are correctly cited. Please note that both of these documents were publicly available in 2008 (on the internet); more recently (2009), they were accepted for publication in the *International Journal of Toxicology*. It is the opinion of the Expert GRAS Panel that the manner in which these documents were made available on the internet, qualifies them as 'publicly available'. Therefore, at the direction of the Expert Panel, these two references (and the two references listed in Clarification #2), are listed when they first became publicly available (i.e. 2008) and secondly, by the acceptance date for IJT.

Clarification #4:
Page 51, last sentence of the second paragraph states: "The primary capsinoids, capsiate, dihydrocapsiate, and nordihydrocapsiate are present in proportions of approximately 7:2:1." Please provide a reference for the proportions of the 3 capsinoids present in CH-19 sweet extract.

Response to #4:
This information is published and was previously submitted to FDA; please refer to the reference list in the GRN 000312 submission (Kodama, et al. (2008a) *Intl. J. Tox.* 27 (S3):13, Kodama, et al. (2008b) *Intl. J. Tox.* 27 (S3): 31 and Bernard, et al. (2008c) *Intl. J. Tox.* 27 (S3): 43).

Please let us know if you have any questions.

Most Sincerely,

Bruce K. Bernard, Ph.D.
President, SRA International Inc.
Authorized Representative for Ajinomoto USA, Inc.

AM

From: Bruce Bernard [mailto:bernard@sra-intl.com]
Sent: Wednesday, March 31, 2010 9:19 AM
To: Harry, Molly *
Subject: GRN 000312: Responses to Question
Importance: High

Dear Ms. Harry,

Per your requested, I am providing the following written answers to your inquiry of 29 March 2010. These are the same answers which were provided verbally during our call of 30 March 2010.

1. I apologize for not discussing the reference we provided to you; however, your email asked for a reference and that is what was provided.
2. You have correctly identified the data (tabular) in the reference we provided upon which the ratio (7:2:1) cited in the GRAS Notification was based.
3. You are correct regarding the data cited in Kobata et al., 1999; therein the ratio is identified as 5:3:1.
4. The explanation of the above 'apparent discrepancy' (2. vs. 3. above) is as follows. In the 11+ years since the data from Kobata et al. 1999 was generated, the methodology has been modified; the resulting methodology was employed for individual 'batch' analyses and resulted in the ratio cited (7:2:1). Thus, the difference was due solely to differences in analytical methodology.
5. I understand that you put no safety significance to a ratio of 5:3:1 versus 7:2:1; you just wanted to understand the basis for the difference.

I would appreciate receiving confirmation of your receipt of this email.

Thank you for your efforts on our behalf.

Sincerely,

Bruce K. Bernard, Ph.D.
Authorized Representative for
Ajinomoto Co.

AM

From: Bruce Bernard
To: Harry, Molly *;
cc: Gaynor, Paulette M;
Subject: Synthetic Dihydrocapsiate (GRN 000312)
Date: Thursday, May 13, 2010 3:01:44 PM

Dear Ms. Harry,

In answer to your recent inquiry, "Please clarify the specific snack foods that are included in this [Snack foods - popped-low-calorie - low fat and rice cakes] food group"
-

Snack foods – low calorie
54403010 Popcorn, air-popped (no butter or no oil added)
54403060 Popcorn, popped in oil, lowfat, low sodium
54403070 Popcorn, popped in oil, lowfat
54403150 Popcorn, sugar syrup or caramel-coated, fat free
54318500 Rice cake, cracker-type
54319010 Puffed rice cake

Sincerely,

Bruce K. Bernard, Ph.D.
Authorized Representative for Ajinomoto

From: Harry, Molly * [mailto:Molly.Harry@fda.hhs.gov]
Sent: Tuesday, May 11, 2010 3:28 PM
To: Bruce Bernard
Cc: Gaynor, Paulette M
Subject: Synthetic Dihydrocapsiate (GRN 000312)

Dear Dr. Bernard,

On the list of proposed uses of synthetic dihydrocapsiate (on tables 1, 6, 7 in GRN 000312), is listed the food group: "Snack foods - popped-low-calorie - low fat and rice cakes". Please clarify the specific snack foods that are included in this food group.

Thanks.

Molly Harry